**A Thesis in General Surgery**

# COMPARATIVE ANALYSIS OF BILIARY CHOLESTEROL LEVELS IN IRON DEFICIENT AND NON-IRON DEFICIENT PATIENTS OPERATED FOR GALL STONE DISEASE

**Submitted in partial fulfilment of the**
**Requirements for the Degree of**
**M.S General Surgery**
**(Branch I)**


**Kilpauk Medical College**

**TheTamil Nadu Dr. M.G.R Medical University**

**Chennai**

**April 2016**

# DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled "**COMPARATIVE ANALYSIS OF BILIARY CHOLESTEROL LEVELS IN IRON DEFICIENT AND NON-IRON DEFICIENT PATIENTS OPERATED FOR GALL STONE DISEASE**" is a bonafide and genuine research work carried out by me under the guidance of Dr.R. Kannan, M.S., Professor, Department of General Surgery, Kilpauk Medical College, Chennai.

This dissertation is submitted to THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI in partial fulfillment of the requirements for the degree of M.S. General Surgery examination to be held in April 2016.

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This is to certify that the dissertation titled "**COMPARATIVE ANALYSIS OF BILIARY CHOLESTEROL LEVELS IN IRON DEFICIENT AND NON-IRON DEFICIENT PATIENTS OPERATED FOR GALL STONE DISEASE"** is a bonafide research work done by **Dr. AJAY VARUN REDDY T.,** Post Graduate in M.S. General Surgery, Kilpauk Medical College, Chennai under my direct guidance and supervision in my satisfaction, in partial fulfillment of the requirements for the degree of **M.S. General Surgery.**

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The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "Comparative analysis of biliary cholesterol levels in iron deficient and non-iron deficient patients operated for gall stone disease"- For Project Work submitted by Dr.T.Ajay Varun Reddy, MS (General Surgery), PG Student, KMC, Chennai-10.

The Proposal is APPROVED.

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**disease is a** extremely **common** condition **affecting adult population** in **both** 4

males and females. The old saying, that gall stone sufferer is a female who is obese and in her fifties may not always hold

**true, as the disease has been** observed **in women** 1

who are nulliparous/primiparous and also in underweight and thin people[1]. Several etiological factors have been studied in the formation of gall stones. Of late a studies have been directed towards establishing the role of several nutritional trace elements in the pathogenesis of gall stone disease. Cholesterol gallstones are very common women who are multiparous, but the cause for this phenemenon remain unclear. Also, this is the same popluation group prone to chornic iron deficiency anemia. With this as the background, Iron Deficiency is a new and particularly interesting parameter which has been studied lately, but with a few studies showing conflicting results. In experimental data from adult prairie dogs, [2] Iron deficiency alters the

**activity of** sevreal **hepatic enzymes,** eventually **leading to increased gall bladder bile cholesterol saturation and** promation **of** cholesteol **crystal formation. Iron** also **acts as a coenzyme for nitric oxide synthetase (NOS), and that is important for the maintenance of basal gall bladder tone and normal relaxation[** 2

3]. Deficiency in iron

**resulted in altered motility of gall and sphincter of oddi (SO),** thus **leading to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile.** [4] **The** 2

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# LIST OF ABBREVIATIONS USED

| | |
|---|---|
| BDS | Common Bile duct stone |
| BT | Bleeding Time |
| CBD | Common bile duct |
| CCK | Cholecystokinin |
| CT | Clotting Time |
| DBD | Common bile duct dilated |
| ECG | Electra Cardiogram |
| ESWL | Extra corporeal shock wave lithotripsy |
| GB | Gallbladder |
| IDA | Imunodiacetic acid |
| LAP | Laparoscopy |
| LFT | Liver Function Test |
| M | Mass |
| MS | Multiple stone in gallbladder |
| MTBE | Methyl ten butyl ether |
| OCP | Oral contraceptive pill |
| OPD | Out Patient Department |
| PT | Prothrombin Time |
| SG | Stone in gall bladder |
| SGOT | Serum Glutamnic Oxaloacetic Transaminase |
| SGPT | Serum Glutamic Pyruvic Transaminase |
| SS | Solitary Stone in gallbladder |
| TG | Thickening of gallbladder |
| US | Ultrasound |

# ABSTRACT


## BACKGROUND AND OBJECTIVE

Gall Stone disease is a common clinical entity affecting adult population of both sexes. The old saying, that gall stone sufferer is a fat, fertile, female of fifty is only partly true, as the disease has been observed in women after their first delivery and also in underweight and thin people. Several etiological factors have been studied in the formation of gall stones. There has been recent interest in establishing the role of several nutritional trace elements in the pathogenesis of gall stone disease.

Iron Deficiency is a new and particularly interesting parameter which has been studied lately, but with a few studies showing conflicting results. Establishing the role of Serum Iron in the etiology of Gall stones is of special importance in our population group because of the huge prevalence of nutritional deficiencies. It also provides scope for early detection, treatment and risk modification if its role as an etiologic/risk factor is clearly defined.

## AIM:

The study is aimed at establishing the role of iron deficiency in the super saturation of bile with respect to cholesterol, which leads to gall stone formation.

## METHODS

A study was done between December 2014 to September 2015 at the department of General Surgery.50 patients suffering from Cholelithiasis, confirmed by USG, were divided into two groups based on serum iron values. Group A, consists of patients with normal serum iron (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Serum Iron, Biliary Cholesterol and Serum Cholesterol of all the patients was obtained. The Biliary cholesterol levels and Serum cholesterol levels of both the groups was analysed by using a student t-test.

## RESULTS

Out of the 50 Patients, 40 (80%) were female and 10 (20%) were males. The female to male ratio was 4:1. The Biliary Cholesterol values for Group A and B respectively were 754.5 ± 398.3 and 1184.7 ± 405.2 mg/dl. The Biliary Cholesterol levels were significantly higher in the Iron Deficient group (Group B) than compared to the Non-Iron Deficient Group. This result was extremely statistically significant with a p value of <0.0004. Similarly, an independent t-test comparing Serum Cholesterol levels in Non-Iron Deficient (184.8 ± 35 mg/dl) and Iron Deficient subjects (171 ± 49.3 mg/dl) did not find a statistically significant difference. (p=0.2544).

## CONCLUSION:

These results suggest that Iron Deficiency has an association with Biliary Cholesterol Levels.

**KEY-WORDS:** Biliary Cholesterol, Iron Deficiency, Gall Stone Disease

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# INTRODUCTION

# INTRODUCTION

Gall Stone disease is a common clinical entity affecting adult population of both sexes. The old saying, that gall stone sufferer is a fat, fertile, female of fifty is only partly true, as the disease has been observed in women after their first delivery and also in underweight and thin people[1]. Several etiological factors have been studied in the formation of gall stones. There has been recent interest in establishing the role of several nutritional trace elements in the pathogenesis of gall stone disease.[2]

Cholesterol gallstones occur most commonly in multiparous women, but the causes for this phenomenon remain unclear[3]. This same patient population is prone to chronic iron deficiency anemia. With this as the background, Iron Deficiency is a new and particularly interesting parameter which has been studied lately, but with a few studies showing conflicting results. In experimental data from adult prairie dogs,[4] Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gall bladder bile cholesterol saturation and promotion of cholesterol crystal formation. Iron acts as a coenzyme for nitric oxide synthetase (NOS), and that is important for the maintenance of basal gall bladder tone and normal relaxation[5]. It was found that iron deficiency resulted in altered motility of gall and sphincter of oddi (SO), leading to biliary stasis and thus increased cholesterol crystal formation in the gall bladder bile.[6]

Therefore, we tested the hypotheses that iron deficiency would alter hepatic cholesterol metabolism causing increased biliary cholesterol saturation and hence enhance gallstone formation. Establishing the role of Serum Iron in the etiology of Gall stones is of special importance in our population group because of the huge prevalence of nutritional deficiencies. It also provides scope for early detection, treatment and risk modification if its role as an etiologic/risk factor is clearly defined.

The present study was conducted on the randomly selected individuals of the South Indian Population, suffering from gall stone formation, to study the role of iron deficiency anaemia in gall stone formation.

# AIMS & OBJECTIVES

# AIMS OF THE STUDY

The study is aimed at:

**PRIMARY:**

1. Determining the association of ***iron deficiency*** in the ***super saturation of bile*** with respect to cholesterol.

**SECONDARY:**

1. Determining the association of ***iron deficiency*** with ***serum cholesterol levels.***
2. ***Sex and Parity specific distributions*** in the serum iron levels and their correlation with biliary cholesterol levels

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

## 3.1. BACKGROUND

Cholelithiasis, is a medical term that literally means stones in the Gall Bladder. Gallstones are solid precipitants that form in the biliary tract, most commonly in the Gall Bladder, though its presence can be in any part of the biliary tract which includes the Common bile duct (referred to as Choledocholithiasis) and also the intra hepatic biliary radicles.

Most cases of Gall Stones develop insidiously and may remain asymptomatic for a long time[7]. The principle etiological factors leading on to the development of Gall Stones are varied and have been extensively studied. These etiological factors also vary according to the type of gall stones with respect to their composition. These will be dealt with, in detail, in the following review.

The principle cause of concern in cases of Gall Stone disease is that a percentage of these patients may develop symptoms typically referred to as Biliary colic. This is mostly due to the migration and blocking of the cystic ductal outflow by the gall stone. If this process persists for more than few hours, an acute inflammation of the gall bladder sets in which is referred to as Acute Cholelithiasis[8].

There is a Chronic phase of Cholecystitis which results due to repeated or subacute inflammation, which predominantly shows features of progressive fibrosis and a dysfunctional Gallbladder[8].

There are many imaging modalities that are used to diagnose Gall Stones, the most common being Ultrasonography[9]. Likewise, the treatment depends on the timing and severity of presentation.

This Review of Literature begins with a Historical note on the problem of Gall Stones. The Anatomy and Physiology of Gallbladder is discussed. An epidemiological picture of the burden of this disease is stated. The discussion turns towards the pathophysiology of gallstones including the various types, and all the etiological/risk factors are discussed in detail. There is particular mention of the role of dietary micronutrients especially iron and its mechanism in formation of gallstones.

From a clinical perspective, the typical presentation, work-up including relevant investigations and imaging modalities are discussed. Lastly, the management of Gallstone disease both from a medical and surgical perspective, including risk factor modification is dealt with.

## 3.2. HISTORICAL NOTE

Gallstones disease has been prevalent even since time of ancient civilizations. There have been records of gall stones found even in Egyptian mummies. This confirms that cholelithiasis has plagued mankind for over 2000 years[10].

Gordon Taylor (1937) suggested that the first clinical description of gallstone disease was recorded in the 4th century BC. Despite description of liver and gallbladder, recognition of the gallstones was not recorded until 5th century. The Greek physician Trallianus studied and reported stones within radicles of a human liver [11].

Vesalius and Fallopius later noted gallstones in dissected human bodies[12]. Joenisius was credited for the first successful cholecystolithotomy in 1676, but the

apparently extracted gallstones from a biliary fistula of the abdominal wall following spontaneous drainage of the abscess[13].

Cholecystotomy was reported and recommended by Jean- Louis Petit in 1743 after he had mistakenly opened the gall bladder when attempting to drain what he thought was an abdominal wall abscess[14].

Nonetheless, the treatment for symptomatic gallstone disease remained relatively primitive and ineffective until the late 1800's. As surgical techniques began to evolve, John Bobbs, an Indian surgeon and others attempted to perform cholecystolithotomy, removing the stone from the gallbladder and leaving the organ in situ[15]. This proved to be effective in ameliorating acute symptoms, physicians were disappointed by the recurrence of symptoms in many of these patients.

In 1882, Langenbuch performed the first successful cholecystectomy, setting the path for therapeutic intervention in cholelithiasis[16].

For the next 100 years open cholecystectomy was the gold standard for definitive management of patients with symptomatic gall stones[17], until the advent of laparoscopy.

### 3.2.1 RISE OF LAPAROSCOPIC CHOLECYSTECTOMY:

Although these advances had widespread rise of laparoscopy for diagnostic and stabilization procedures in gynecological surgery, few general surgeons used it on their surgical practice. The exceptions were pioneering individuals such as George Berci and Alfred Cushiri who used diagnostic laparoscopy for everlasting and staging patients with abdominal malignancies.

In 1987, Philippe Mouret performed the first laparoscopic cholecystectomy in a human[18]. Almost simultaneously Mc Kernan and Saye performed the first

laparoscopic cholecystectomy in the United States in 1988[19].

In fact, in 1985, Prof. Erich Muhe of Boblingen, Germany had carried out the first laparoscopic cholecystectomy. He presented his technique at the Congress of the German Surgical Society[20].Unfortunately, his technique was not appreciated by his colleagues and did not become popular. His work was not realized until 1999, when he was recognized by SAGES for having performed the first laparoscopic cholecystectomy.

The first laparoscopic cholecystectomy in India was performed in 1990 at the JJ Hospital, Mumbai, followed by few months later in Pune by Dr. Jyotsna Kulkarni[21].Within a short span of five years laparoscopic cholecystectomy has surpassed conventional cholecystectomy as procedure of choice for diseases of gallbladder[22].

Thus a good amount of evidence is available that man in various areas of the world has suffered from calculous biliary tract disease even before the era when history was first recorded. The current treatment of this condition which, now accepted to be surgical is a development of the last 100 years[23]. The control and/or prevention of this pathological condition in the future is to be expected. The general course of events in man's conquest of his diseases has followed a rather consistent pattern. First, there has been the discovery and description of pathology; second, the definition of the alterations of physiology; and third, the correlation of these with the clinical manifestations and the complaints and symptoms of the patient.[11]

## 3.3 EMBRYOLOGY[24-26]

At 3rd week when the embryo is 3mm in length an endodermal bud arises from the ventral aspect of the gut at the point between for foregut and midgut. This endodermal bud enlarges and divides into pars hepatica and pars cystica. It passes through the septum transversum and grows into ventral mesogastrium. Cranial portion that is pass hepatica and caudal portion pass cystica.

- Pars cystica develops into gall bladder and cystic duct.
- Pars hepatica cells grows into the transverse septum
- At 12th week of gestation liver function starts and cystic duct joins the hepatic duct and forms common bile duct (CBD).

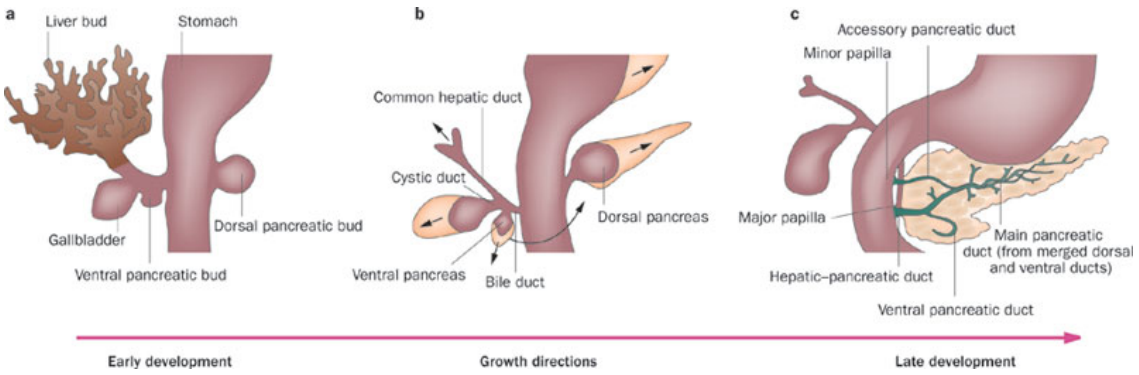



**Figure 1. Embryological Development**

## 3.4 ANATOMY OF THE BILIARY SYSTEM

The anatomy of the biliary tract can be divided into various segments, including the intrahepatic ducts, the extrahepatic ducts, the gallbladder and cystic duct, and the sphincter of Oddi[27].

### 3.4.1 Intrahepatic Ducts

The anatomy of the intrahepatic ducts is intimately associated with the anatomy of the liver. The lobar and segmental anatomy of the liver is determined by the sequential branching of the portal vein, hepatic artery, and biliary tree as they enter the parenchyma at the hilum[28]. All three of these structures follow roughly parallel courses and bifurcate just before entering the liver. This major bifurcation divides the liver into left and right lobes. According to Couinaud's classification[29], the caudate lobe is segment I; segments II to IV are on the left; and segments V to VIII are on the right.

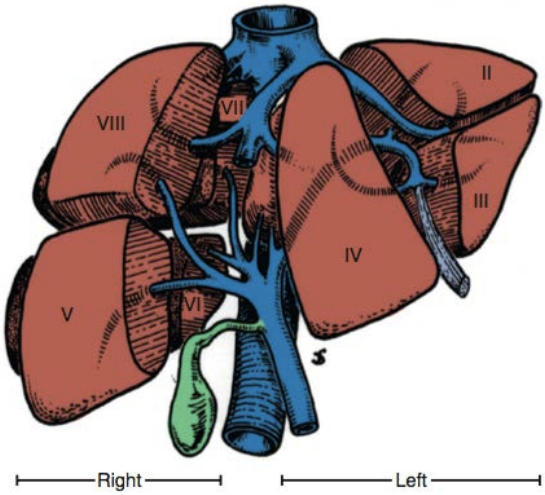



**Figure 2. Segmental biliary drainage of the liver**

The biliary drainage of the right and left liver is into the right and left hepatic ducts, respectively. The left hepatic duct is formed within the umbilical fissure from the union of the three segmental ducts draining the left side of the liver (segments II through IV). The left hepatic duct crosses the base of segment IV (medial segment of the left lobe) in a horizontal direction to join the right hepatic duct and form the common hepatic duct. The right hepatic duct drains

segments V through VIII and is formed from the union of the right posterior and right anterior segmental ducts. The right posterior segmental duct is formed by the confluence of ducts draining segments VI and VII. The posterior segmental duct initially courses in a nearly horizontal direction before descending in a more vertical direction to join the anterior segmental duct. The right anterior segmental duct is formed by the union of the ducts draining segments V and VIII. In approximately 15% to 20% of cases, the right posterior duct drains into the left hepatic duct.[30] The biliary drainage of the caudate lobe (segment I) is variable.[31] In approximately 80% of the individuals, the caudate lobe drains into both the right and left hepatic ducts. In 15% of cases, the caudate lobe drains only into the left hepatic duct, and in the remaining 5% of cases, the caudate is drained exclusively by the right hepatic duct.[32]

### 3.4.2 Extrahepatic Ducts

Most patients have a bifurcation where the right and left hepatic ducts join to form the common hepatic duct. This junction may occur as a wide or an acute angle, or the two hepatic ducts may run parallel to each other before joining. In some patients, three hepatic ducts join to form the common hepatic duct. Usually, the hepatic ducts meet just outside of the liver parenchyma, with the cystic duct entering 2 to 3 cm distally[33]. Occasionally, the two hepatic ducts do not unite until after the cystic duct has joined the right hepatic duct. The common hepatic duct extends for a variable length from the junction of the right and left hepatic ducts to the entrance of the cystic duct into the gallbladder.

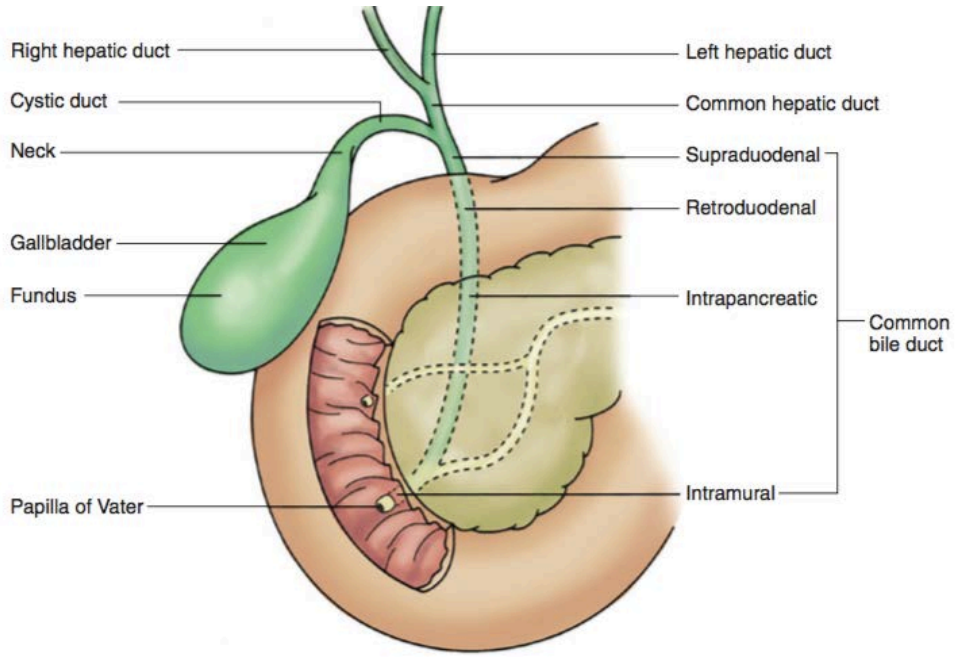



**Figure 3. Anatomic divisions of gallbladder and extrahepatic biliary tree.**

The common bile duct is formed by the union of the cystic and common hepatic ducts. The common bile duct is approximately 8 cm in length, but, like the hepatic duct, it varies in length according to the point of union of the cystic duct and the common hepatic duct. The normal diameter of the common bile duct ranges from 4 to 9 mm. The common bile duct is considered enlarged if the duct diameter exceeds 10 mm. The upper third, or supraduodenal portion, of the common bile duct courses downward in the free edge of the lesser omentum, anterior to the portal vein and to the right of the proper hepatic artery. The middle third, or retroduodenal portion, of the common bile duct passes behind the first portion of the duodenum, lateral to the portal vein and anterior to the inferior vena cava. The lower third, or intrapancreatic portion, of the common bile duct traverses the posterior aspect of the pancreas in a tunnel or groove to enter the second portion of the duodenum, where it is usually joined by the pancreatic duct.

The intramural or intraduodenal portion of the common bile duct passes obliquely through the duodenal wall to enter the duodenum at the papilla of Vater[27].

The relationship between the lower common bile duct and pancreatic duct is variable[34]: (1) the two structures may rarely unite outside the duodenal wall to form a long common channel; (2) the bile duct and pancreatic duct usually join within the duodenal wall to form a short common channel; or (3) the two structures may rarely enter the duodenum independently through separate orifices. The lower portion of the common bile duct and the terminal portion of the pancreatic duct are enveloped and regulated by a complex sphincter, the sphincter of Oddi. In 5% to 10% of patients who have pancreas divisum, the dorsal pancreatic duct enters the duodenum through an accessory sphincter, whereas the ventral pancreatic duct joins the common bile duct at the sphincter of Oddi.

The extrahepatic bile ducts contain a columnar mucosa surrounded by a connective tissue layer. The surface is relatively flat, with basal nuclei and an absent or small nucleolus. The lamina propria consists of collagen, elastic fibers, and vessels. Occasional lymphocytes are found, and pancreatic acini and ducts may be seen in the wall of the intrapancreatic portion of the distal common bile duct. Muscle fibers in the bile duct are sparse and discontinuous. The muscle fibers that are present are usually longitudinal, although occasional circular fibers are observed. The distal common bile duct begins to develop a more substantial muscle layer in the intrapancreatic portion of the duct, which becomes prominent at the sphincter of Oddi, where distinct bundles of longitudinal and circular fibers are clearly identified.

### 3.4.3 Gallbladder and Cystic Duct

The gallbladder is a pear-shaped organ that lies on the inferior surface of the liver at the junction of the left and right hepatic lobes between Couinaud's segments IV and V .[35] The gallbladder varies from 7 to 10 cm in length and from 2.5 to 3.5 cm in width. The gallbladder's volume varies considerably, being large during fasting states and small after eating. A moderately distended gallbladder has a capacity of 50 to 60 mL of bile but may become much larger with certain pathologic states. The gallbladder has been divided into four areas: the fundus, body, infundibulum, and neck. Hartmann pouch is an asymmetric bulge of the infundibulum that lies close to the gallbladder's neck. The neck points in a cephalad and dorsal direction to join the cystic duct[36].

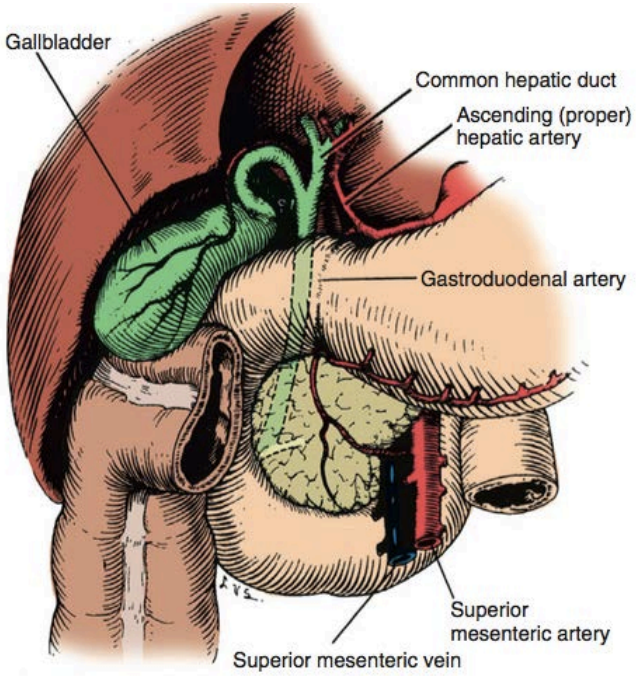



**Figure 4. Anatomic relations of gallbladder.**

The gallbladder wall consists of five layers[37]. The inner- most layer is the epithelium, and the other layers are the lamina propria, smooth muscle,

perimuscularsubserosal connective tissue, and serosa. The gallbladder has no muscularis mucosae or submucosa. Most cells in the mucosa are columnar cells, and their main function is absorption, but they also are capable of active secretion.[38] These cells are aligned in a single row, with slightly eosinophilic cytoplasm, apical vacuoles, and basal or central nuclei.

The lamina propria contains nerve fibers, vessels, lymphatics, elastic fibers, loose connective tissue, and occasional mast cells and macrophages. The muscle layer is a loose arrangement of circular, longitudinal, and oblique fibers without well-developed layers. Ganglia are found between smooth muscle bundles. The subserosa is composed of a loose arrangement of fibroblasts, elastic and collagen fibers, vessels, nerves, lymphatics, and adipocytes.

Rokitansky-Aschoff sinuses are invaginations of epithelium into the lamina propria, muscle, and subserosal connective tissue[39]. These sinuses are present in approximately 40% of normal gallbladders and are present in abundance in almost all inflamed gallbladders. The ducts of Luschka are tiny bile ducts found around the muscle layer on the hepatic side of the gallbladder. They are found in approximately 10% of normal gallbladders and have no relation to the Rokitansky-Aschoff sinuses or to cholecystitis.

The cystic duct arises from the gallbladder and joins the common hepatic duct to form the common bile duct. The length of the cystic duct is variable, averaging between 2 and 4 cm. The cystic duct usually courses downward in the hepatoduodenal ligament to join the lateral aspect of the supraduodenal portion of the common hepatic duct at an acute angle.[30] Occasionally, the cystic duct may

join the right hepatic duct, or it may extend downward to join the retroduodenal duct[40]. In addition, the cystic duct may join the common hepatic duct at a right angle, may run parallel to the common hepatic duct, or may enter the common hepatic duct dorsally, on its left side, behind the duodenum, or, rarely, may enter the duodenum directly. The cystic duct contains a variable number of mucosal folds, similar to those found in the neck of the gallbladder. Although referred to as valves of Heister, these spiral folds do not have a valvular function. Variations in the length and course of the cystic duct and its point of union with the common hepatic duct are common.

In 1891, Calot described a triangular anatomic region formed by the common hepatic duct medially, the cystic duct laterally, and the cystic artery superiorly.[41]Calot triangle is considered by most to comprise the triangular area with an upper boundary formed by the inferior margin of the right lobe of the liver, rather than the cystic artery.[42] A thorough appreciation of the anatomy of Calot triangle is essential during perfor- mance of a cholecystectomy because numerous important structures pass through this area. In most instances, the cystic artery arises as a branch of the right hepatic artery within the hepatocystic triangle. A replaced or aberrant right hepatic artery arising from the superior mesenteric artery usually courses through the medial aspect of the triangle, posterior to the cystic duct.

Aberrant or accessory hepatic ducts also may pass through Calot triangle before joining the cystic duct or common hepatic duct. During performance of a cholecystectomy, clear visualization of the hepatocystic triangle is essential with accurate identification of all structures within this triangle.

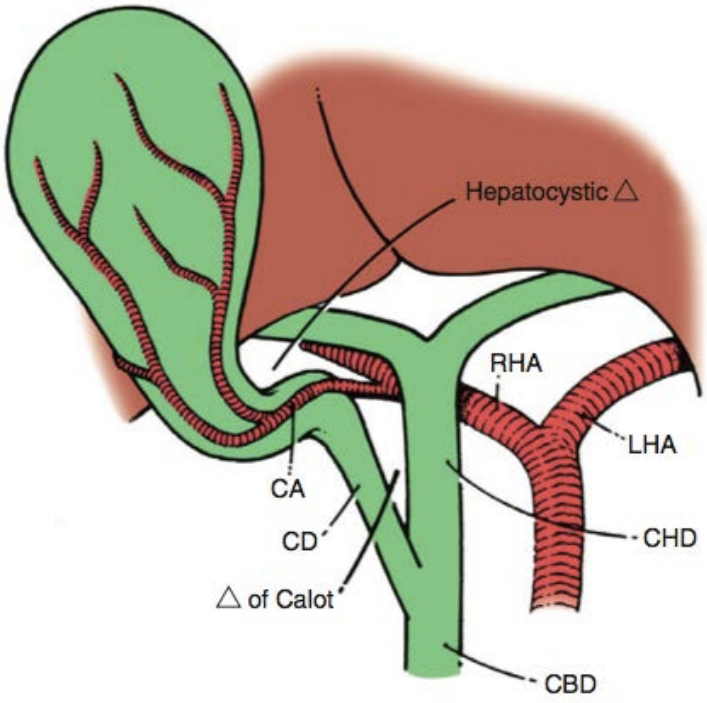



**Figure 5. The triangle of Calot and the hepatocystic triangle.**

### 3.4.4 Sphincter of Oddi

The entire sphincteric system of the distal bile duct and the pancreatic duct is commonly referred to as the sphincter of Oddi. This term is imprecise because the sphincter is subdivided into several sections and contains both circular and longitudinal fibers. The sphincter mechanism functions independently from the surrounding duodenal musculature and has separate sphincters for the distal bile duct, the pancreatic duct, and the ampulla. In more than 90% of the population, the common channel, where the biliary and pancreatic ducts join, is less than 1.0 cm in length and lies within the ampulla. In the rare situation in which the common channel is longer than 1.0 cm or the biliary and pancreatic ducts open separately into the duodenum, pathologic biliary or pancreatic problems are likely to develop. The entire sphincter mechanism is actually composed of four

sphincters containing both circular and longitudinal smooth muscle fibers. The four sphincters are the superior and inferior sphincter choledochus, the sphincter pancreaticus, and the sphincter of the ampulla.[43]

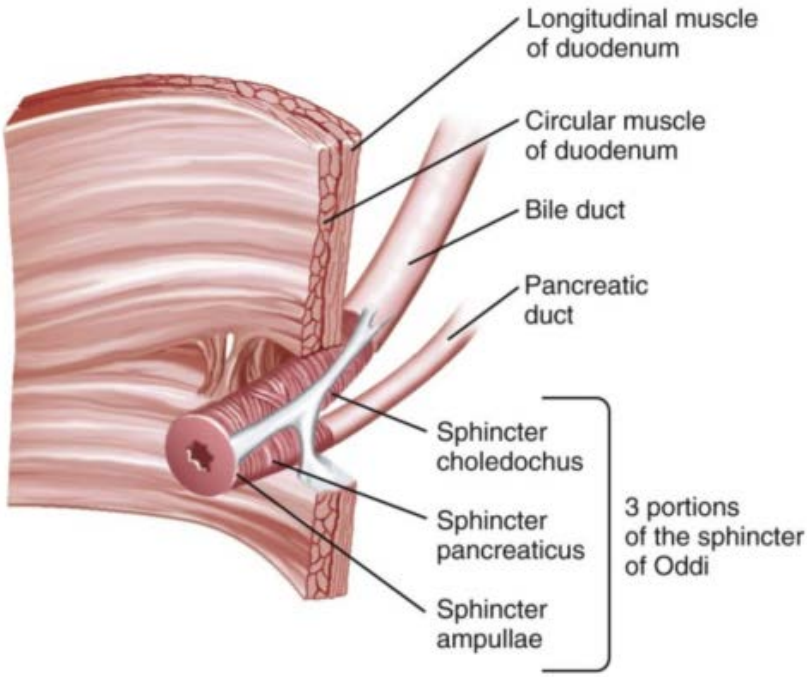



**Figure 6. Anatomy of the Sphincter of Oddi**

### 3.4.5 Blood Supply

Major blood supply is from cystic artery which is branch of right hepatic artery. It runs in Calot's triangle closed to cystic duct. At the superior border of the neck of the gallbladder it divides into superficial and deep branches. Occasionally cystic artery may arise from hepatic artery proper or rarely from gastroduodenal artery. Cystic artery also supplies branches to hepatic ducts and upper part of common bile duct. Venous drainage is carried out by small veins which enter directly liver[44].

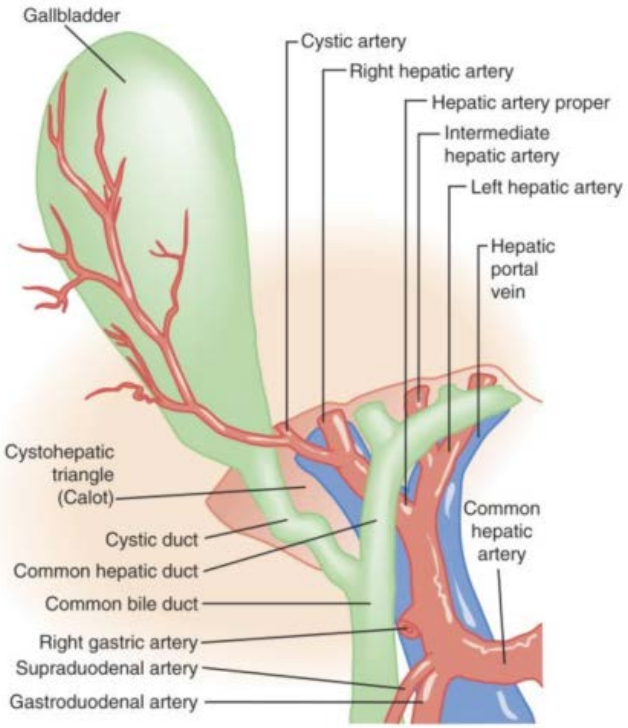



**Figure 7: Arterial blood supply of the gall bladder**

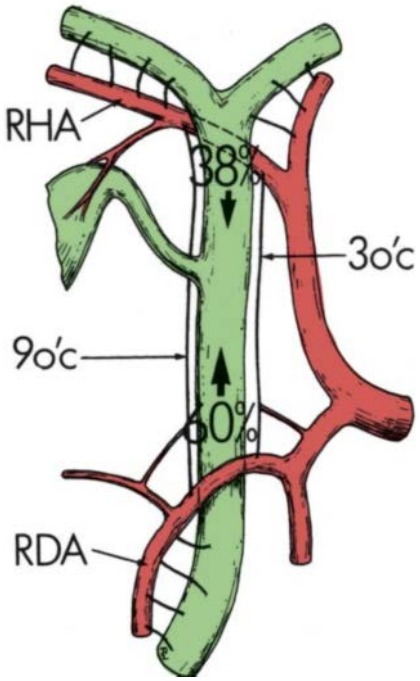



**Figure 8: Arterial blood supply of the extrahepatic biliary tree**

### 3.4.6 Lymphatics

Proximally the lymphatic channels of the gallbladder communicate with those of Glisson‘s capsule of the liver which in turn drain into the thoracic duct through several channels. Distally the lymphatics from gallbladder and extrahepatic bile duct drain into the cystic lymph node, which is situated near the cystic artery origin from the right hepatic artery[45].

### 3.4.7 Nerve Supply

Parasympathetic fibres of hepatic branch of anterior vagal trunk stimulate contraction of gallbladder and relax ampullary sphincter. Sympathetic fibres from cell bodies of coeliac ganglion inhibit contraction of gallbladder. The hormonal activity is much more important than neural function.

Afferent pain fibres pass mainly through the right sympathetic fibres into the spinal segments T7-T9. This causes referred pain over the right infrascapular region. Some fibres may pass through the right phrenic nerve, C3-C5.

Fibers from the right phrenic nerve travel by way of the phrenic, celiac, and hepatic plexuses to reach the gallbladder. Many of these fibers are afferent and may account for the pain referred to the right hypochondrium and radiating the back between the shoulder blades in some patients with gallbladder diseases[46].

Burnett and associates demonstrated three nerve plexuses: subserous, muscular, and mucosal. The ganglion cells in each nerve plexus decrease in number from subserous to mucosal levels. In comparison with the myenteric plexus of the gut, the subserous plexus ganglia are larger and spaced farther apart.

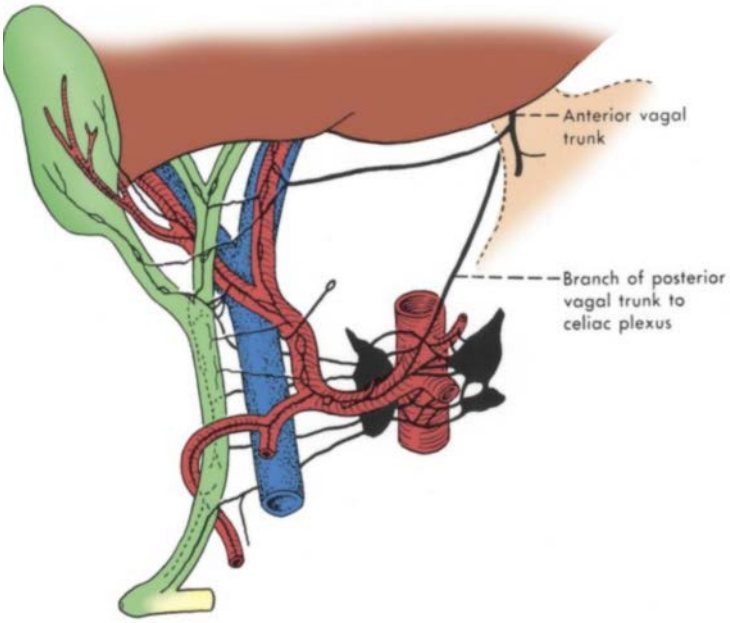



**Figure 9: Nerve supply to the Extrahepatic Bile Tree.**

### 3.4.8 Anomalies of Gallbladder

- The gallbladder may be septate, transversely or longitudinally placed.
- The gallbladder may be double with a single cystic duct.
- The gallbladder may be double with separate ducts opening into hepatic common or both ducts. The serosa may be separate or common.
- Small ducts may connect gallbladder with liver. Usually these become obliterated.
- The folded fundus (Phrygian cap) deformity is the commonest congenital abnormality of the gallbladder. It has no pathological significance, but when present it can be seen on cholecystectomy.

## ANOMALIES OF THE GALLBLADDER[47]

| | |
|---|---|
| **FORMATION** | ➢ Phrygian cap<br>➢ Bilobed gallbladder<br>➢ Hourglass gallbladder.<br>➢ Diverticulum of the gallbladder.<br>➢ Rudimentary gallbladder |
| **NUMBER** | ➢ Absence of the gallbladder (agenesis)<br>➢ Duplication of the gallbladder |
| **POSITION** | ➢ Floating gallbladder<br>➢ Intrahepatic gallbladder<br>➢ Left-sided gallbladder<br>➢ Transverse gallbladder<br>➢ Retrodisplaced gallbladder |

## ANOMALIES OF GALLBLADDER

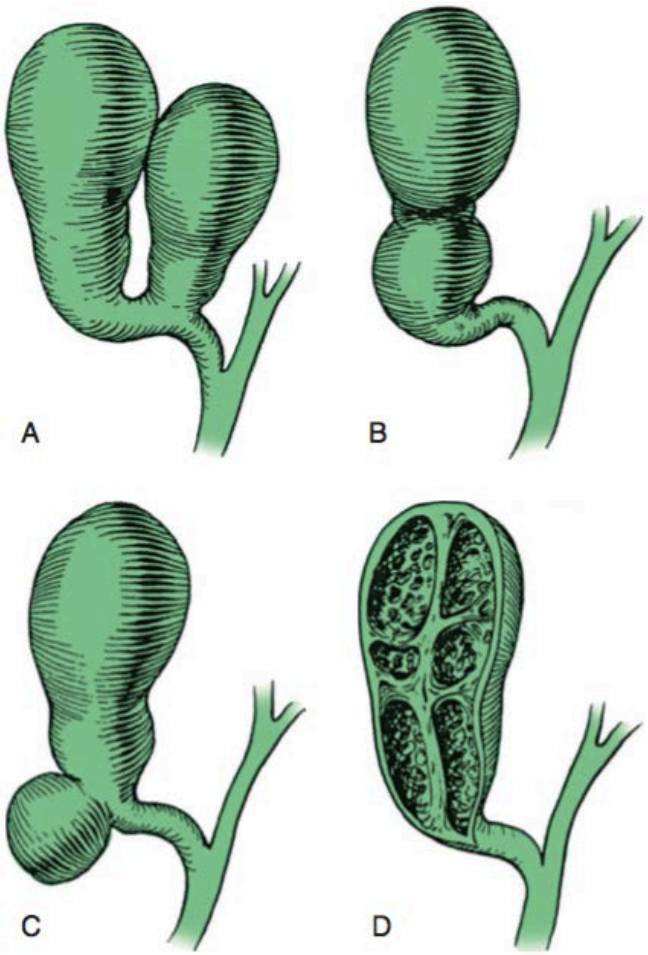



**Figure 10** - Anomalies of the gallbladder. A, Bilobed gallbladder. B, Hourglass gallbladder. C, Congenital diverticulum of the infundibulum. D, Septate gallbladder.

### 3.4.9 Variations of Cystic Duct Anatomy

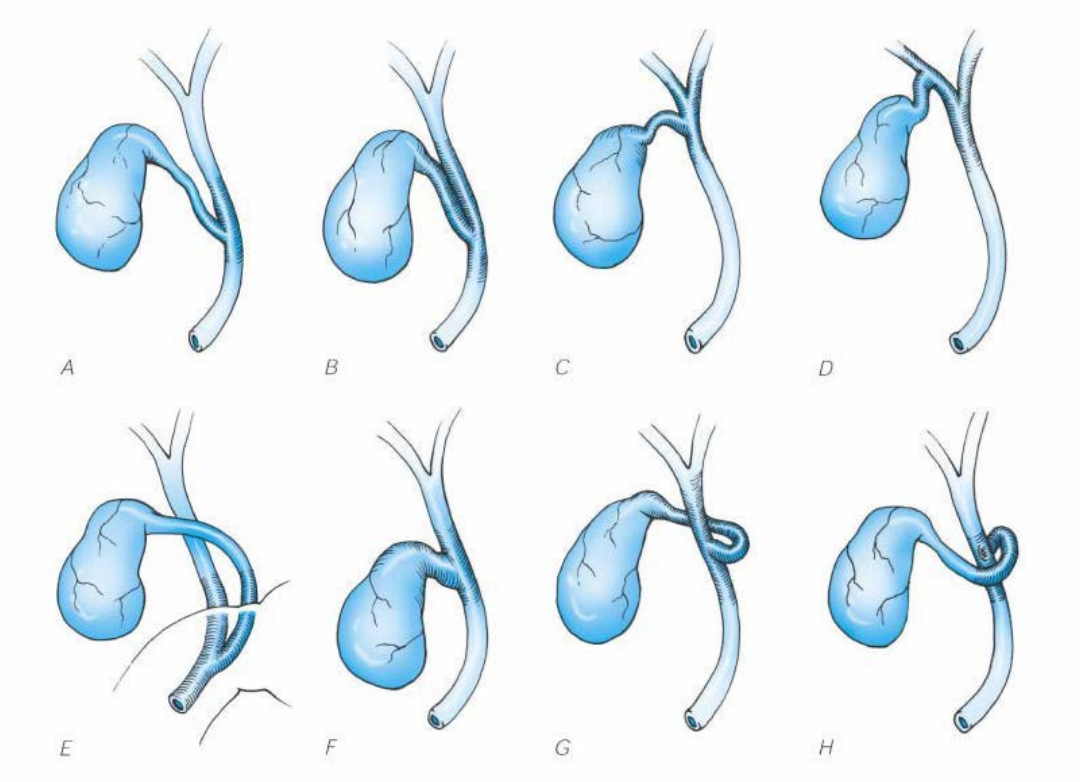



**Figure 11[48].**

A. Low junction between the cystic duct and common hepatic duct.

B. Cystic duct adherent to the common hepatic duct.

C. High junction between the cystic and the common hepatic duct.

D. The cystic duct drains into right hepatic duct.

E. Long cystic duct that joins the common hepatic duct behind the duodenum.

F. Absence of the cystic duct.

G. The cystic duct crosses posterior to the common hepatic duct and joins it anteriorly. H. The cystic duct courses anterior to the common hepatic duct and joins it posteriorly.

### 3.4.10 Variations in the Arterial supply to the Gallbladder

Variations in the arterial supply of the extrahepatic biliary tree are more common than variations in the ductal anatomy. Anatomic variations of the hepatic and cystic arteries are present in approximately 50% of individuals[32,49,50]. Based on their anatomic dissections, Benson and Page described three surgically important variations in the arterial anatomy.[49] An accessory or double cystic artery occurs in approximately 15% to 20% of individuals.[49,51] These arteries usually arise from the right hepatic artery within Calot triangle. Triple cystic arteries are unusual and occur in less than 1% of individuals. During dissection of Calot triangle, care should be taken to exclude the presence of an accessory cystic artery[52].

In 5% to 15% of individuals, the right hepatic artery courses through Calot triangle in close proximity to the cystic duct before turning upward to enter the hilum of the liver.[49,50]In this location, the cystic artery arises from the convex aspect of the angled or humped portion of the hepatic artery. This “caterpillar hump” right hepatic artery may easily be mistaken for the cystic artery and may be inadvertently ligated during performance of a cholecystectomy. The cystic artery that arises from the caterpillar hump is typically short and may easily be avulsed from the hepatic artery if excessive traction is applied to the gallbladder.[49]

The cystic artery may occasionally pass anterior to the common bile duct or common hepatic duct.[53] In this location, the cystic artery, rather than the cystic duct, is usually the first structure encountered during dissection of the lower

border of Calot triangle.[51] These arteries usually require ligation and division early in the dissection during a cholecystectomy, to provide adequate exposure of the cystic duct.

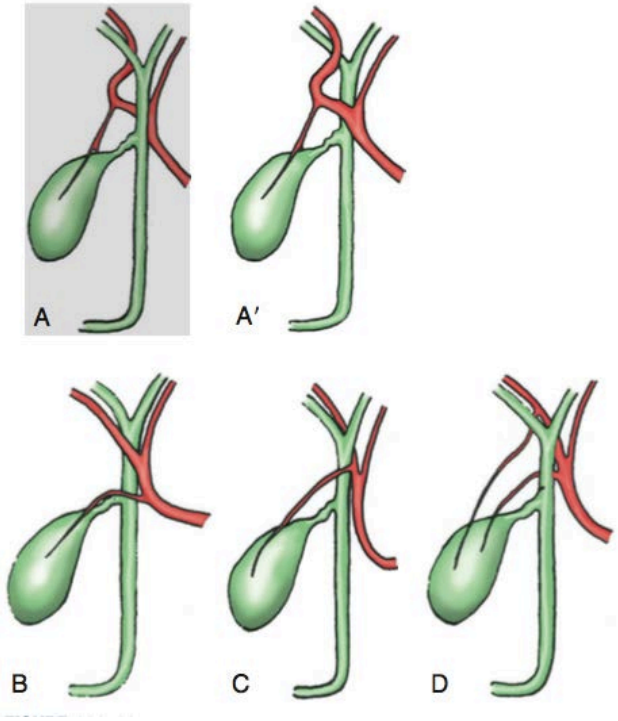



**Figure 12** . Vascular anomalies. A, A′, “Caterpillar hump” right hepatic artery. B, Right hepatic artery anterior to common hepatic (or common bile) duct. C, Cystic artery anterior to common hepatic (or common bile) duct. D, Accessory cystic artery.

## 3.5 PHYSIOLOGY OF GALLBLADDER

Bile secretion by liver is an active and continuous process. Its expulsion into duodenum, which is its site of action, is intermittent. Hence, it is necessary for bile to be stored and to be released when needed. Gallbladder serves this main function. Strictly bile is not a digestive secretion, because it doesn't possess any digestive enzymes. Liver secretes bile at the rate of 40ml/hr. The sphincter of Oddi dictates the flow of bile[54].

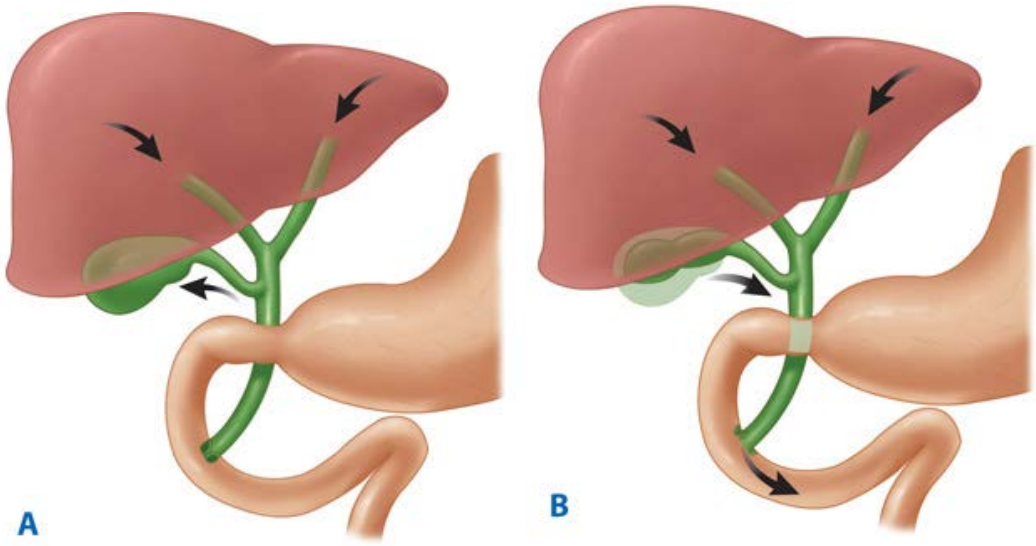



**Figure 13:** The effect of cholecystokinin on the gallbladder and the Sphincter of Oddi.

A. During fasting, with the sphincter of Oddi contracted and the gallbladder filling.

B. In response to a meal, the sphincter of Oddi relaxed and the gallbladder emptying.

The functions of gallbladder are –

- Reservoir of bile
- Concentration of bile
- Pressure regulation
- Secretion of bile

### 3.5.1 Mechanism of storage

The CBD is shut off from duodenum by sphincter of Oddi when pressure exceeds >70 mm H2O, bile is directed from CBD into gallbladder. Because of inherent capacity of gallbladder to absorb water and inorganic constituents, bile is concentrated 4-10 times.

### 3.5.2 Movements of gallbladder[16]

- Tonic contractions begin 5-30 minutes after food intake, intermittently till the gallbladder is empty. Normal emptying time varies between 2-5 hours
- Rhythmic contractions, which are weak, not exceeding 50mmH2O are not ableto expel bile into duodenum. Since this pressure is less than the secretory pressure of liver, filling and evacuation is entirely dependant upon reciprocal sphincter of Oddi contraction and relaxation.

### 3.5.3 Mechanism in expulsion of bile[55]

Expulsion of bile requires 2 factors

- increased pressure of bile
- relaxation of sphincter of Oddi.

Pressure of bile is increased and secretion of bile is stimulated by bile acids and fatty meal. Gallbladder contraction is brought about by stimulation of right vagus,

which is motor to gallbladder and inhibitory to sphincter. Thesecond mechanism is hormonal which is more important than neural reflex. Cholecystokinin is secreted by duodenal mucosa, in response to food and low pH. The hormone has potent stimulative action on gallbladder and inhibitory action on sphincter of Oddi.

### 3.5.4 Bile salts and Bile acids

These are steroid molecules, formed from cholesterol by hepatocytes and are major pathway of cholesterol excretion by body. To enhance their solubility in bile, bile acids are conjugated with glycine and taurine before excretion as sodium salts.

- Primary bile acids - cholic acid / cheno acid
- Secondary bile acids - deoxycholic / lithocholic / 7 ketolithocholic acid
- Tertiary bile acids -ursodeoxycholic acid

### 3.5.5 Bile Pigments

Bilirubin is the chief bile pigment, produced by the breakdown of senescent RBCs in reticuloendothelial system. Biliverdin is produced from bilirubin.

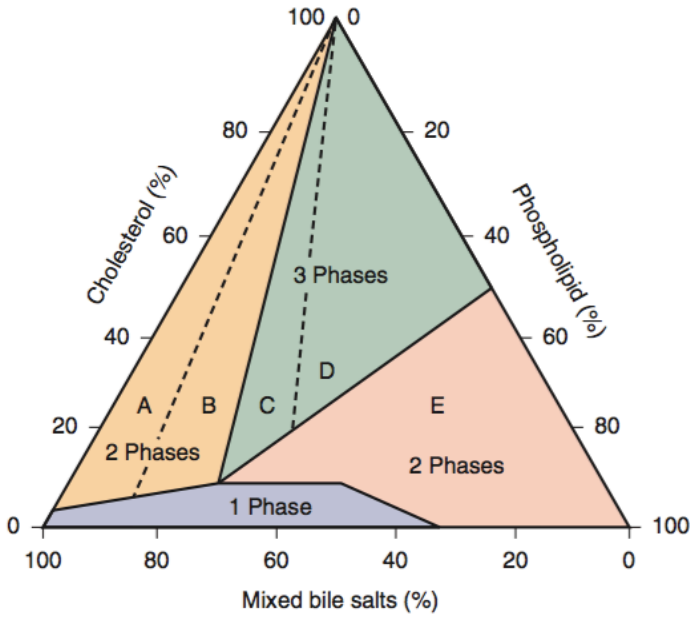



**Figure 14**[16]**.** Equilibrium phase diagram of a cholesterol– phospholipid (lecithin)–mixed bile salt system (37°C, 0.15 M NaCl, pH 7.0, total lipid concentration 7.5 g/dL) showing positions and configuration of crystallization regions. Components are expressed in moles percent.

The 1-phase micellar zone at the bottom is enclosed by a *solid angulated line*, and above it, *2 solid lines* divide the two-phase zones from a central 3-phase zone. Based on the solid and liquid crystallization sequences present in the bile, the left two-phase and central three-phase regions are divided by *dashed lines* into regions A to D. The number of phases given represents the equilibrium state. The phases are cholesterol monohydrate crystals and saturated micelles for crystallization **regions A and B;** cholesterol monohydrate crystals, saturated micelles, and liquid crystals for **regions C and D;** and liquid crystals of variable composition and saturated micelles for **region E.**

## 3.6 GALLSTONE DISEASE

### 3.6.1 Epidemiology*:*

Gallstones are the most common biliary pathology. The incidence of biliary calculous disease varies widely throughout the world. By the age of 75, about 35% of women and 20% of men would have developed gallstones. The incidence of gallstone disease in Asia is considerable and constitutes a problem of enormous magnitude. The incidence of cholesterol gallstones is increasing in Asia for the reasons that may be related to environmental and dietary considerations.

Most patients with gallstones are asymptomatic and only about 10% will have developed symptoms five years after discovery. In a functioning gall bladder, most of the gall stones are cholesterol stones. Gall stone disease is a relatively common problem in our country particularly in North India. It is estimated that more than sixty percent of these patients have cholesterol stones. Recent studies from south India have highlighted pigment and mixed variety of gall stones to be more common (> 90 %) in contrast to cholesterol stones.

In India, there is a dual pattern of prevalence. Some studies have shown that North Indians are more prone to cholelithiasis than South Indians. The nature of the disease is also different in North India and South India. In North India, Cholesterol stones form the majority of gallstones. In contrast to this, pigment stones are more frequent in South India.

**A. True Incidence :** 5-year incidence in women aged 30, 40, 50, and 60 are (4%), (3.6%), (3%) and (3.7%) years and the same incidence rate in men were 0.3%, 2.9%, 2.5% and 3.3% at the same age. This shows that the incidence is more in women.

**B. Prevalence and Incidence :** Gallstones are two times more common in women than in men.

**C. Ethnic Predisposition :** Several genes that are associated with gallstone formation and resistance are identified in mice. The importance of these genes in human gallstone formation has not been established. Pima Indians in southern Arizona are an example of an extremely high risk population in which 70% of women less than 25 years are affected by the disease. Populations at the lowest risk are sub Saharan Africans and Asians.

**D. Risk Factors:** Gallstone disease is multifactorial in origin and occur sporadically. Specific risk factors predisposing gallstones have been identified. These are discussed in detail.

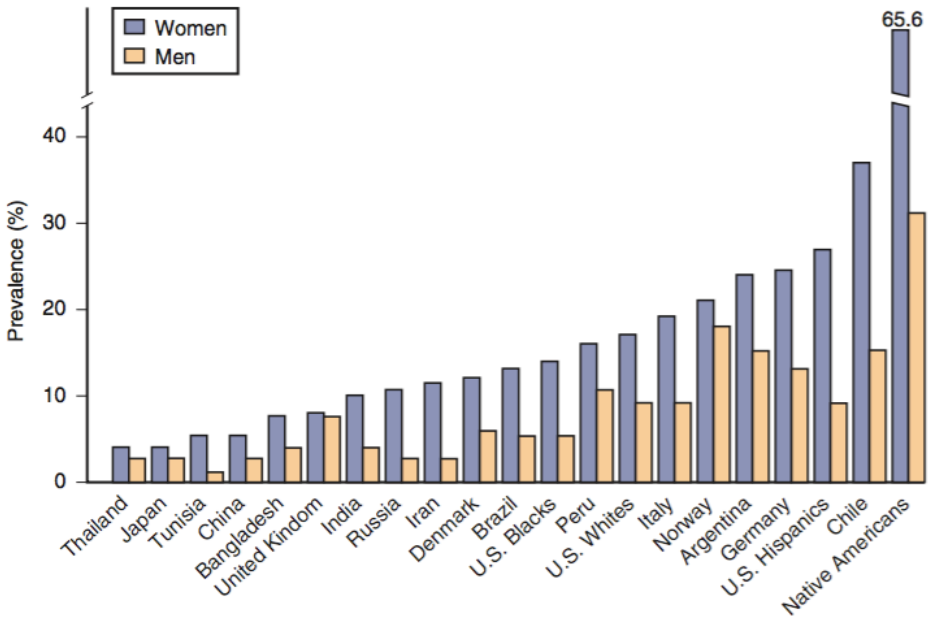



**Figure 15. Prevalence rates of cholesterol gallstones by gender in 18 countries.**

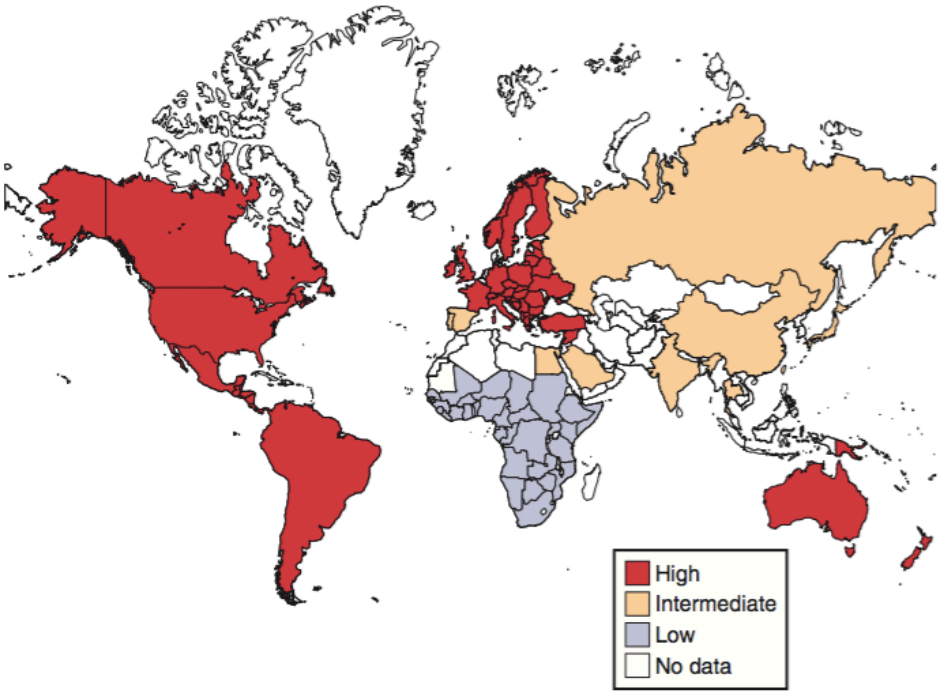



**Figure 16. Prevalence of cholesterol gallstones around the world.**

### 3.6.2 Classification of gall stones[56]

1) Pure gallstones
   - Cholesterol gallstones 70%
   - Pigment gallstones 30%
   - Calcium carbonate gallstones
2) Mixed and combined stones

**Cholesterol Gallstones:[57]**

10% gallstones are cholesterol stones. They are usually solitary with smooth surface, oval or round in shape, pale yellow in colour. They are thought to be formed in aseptic static bile and commonly found in Hartman's pouch. On section they shows radiating lines crossing the circular strata. In combined

gallstone, the stone starts as pure cholesterol stones but ultimately receives mixed covering of pigment and cholesterol.

**Pigment Gallstones:**

May be pure or contain Calcium bilirubinate. They constitute about 80% of all gallstones. They are Dark or black brown in colour, found exclusively in the gallbladder associated with excessive haemolysis like hereditary spherocytosis, sickle cell disease, thalassemia etc. Excessive breakdown of hemoglobin resulting in increase bilirubin which are excreted in bile and forms pigment stones in the gallbladder. Stones are usually appear as small soft fatty like masses.

Calcium bilirubinate stones are brown to orange in colour and soft in consistency. These stones are more often seen in bile ducts. These stones are often caused by infection (E.Coli and parasites).

**Calcium Carbonate Stones:**

Calcium carbonate stones are rarest type of stone they are grayish white in colour with smooth surface or articulated surface. Increase alkalinity of the bile favours this stone formation.

**Mixed or Combined Stones:**

Mixed stones have varying proportion of all three of the stone forming constituents of the bile eg. cholesterol, bile pigment and calcium. They constitute about 10% of gallstones.

Combined stones are those in which central core or external layers are pure and the reminder of the stone is mixture of constituents. Combined stones may be solitary but mixed gallstones are invariably multiple with faceted surface. Stones

may vary in size few cm in diameter. Colour of the stone depends on constituents of stones.

Pale yellow - Cholesterol

Black - Calcium bilirubinate

Grayish white - Calcium carbonate.

**CLASSIFICATION OF GALLSTONES:**

| | **Cholesterol** | **Black** | **Brown** |
|---|---|---|---|
| Location | Gallbladder, ducts | Gallbladder, ducts | Ducts |
| Major constituents | Cholesterol | Bilirubin pigment polymer | Calcium bilirubinate |
| Consistency | Crystalline with nucleus | Hard | Soft, friable |
| % Radio-opaque | 15% | 60% | 0% |
| **Associations** | | | |
| **Infection** | **Rare** | **Rare** | **Usual** |
| Other diseases | | Haemolysis, Cirrhosis | Chronic partial biliary obstruction |

On section of laminated central nucleus may contain epithelial debris and bacteria. This suggests inflammatory origin of stones. Chemical inflammatory changes prepare the soil for bacterial invasion.

### 3.6.3 Risk factors associated with gallstone formation[58]:

1. Cholesterol stones
    - i. Age > 40 years
    - ii. Estrogens
        - a. Female sex (2-3 times the risk in men)
        - b. Pregnancy (risk increases with number of pregnancies)
        - c. Estrogen containing OCPs.
    - iii. Genetic or ethnic variation
    - iv. High fat, low fiber diet
    - v. Obesity
    - vi. Hyperlipidaemia
    - vii. Bile salt loss ( Ileal disease or resection; Crohn's disease )
    - viii. Cystic fibrosis
    - ix. Anti-hyperlipidaemic drugs ( Clofibrate )
    - x. Impaired gall bladder emptying
        - a. Truncalvagotomy
        - b. Type-1 diabetes
        - c. Octreotide
        - d. Total parenteral nutrition
        - e. Starvation or rapid voluntary weight loss
2. Pigment stones
    - i. Haemolytic disease
    - ii. Biliary stasis
    - iii. Biliary infection

### 3.6.4 Description of Risk Factors

**1) Age and gender**

Gallstone disease increases with age. Hence bile become more lithogenic with increasing age. Most studies report that incidence and prevalence of gallstones is three to four fold higher in female. But after 50 years the incidence may become equal in male and females. This may be due to increase oestrogen in young women lead to increased secretion of cholesterol into bile[59].

**2) Pathophysiology of gallstone formation with aging**

Changes in bile composition with aging accounts for an increase risk of cholesterol gallstone formation. Biliary cholesterol saturation index (CSI) rises with age in both men and women. An inverse relation was seen between the age and hepatic bile salt synthesis and activity of enzyme 7 α-hydroxylase (rate limiting enzyme for bile salt synthesis).

Factors that change with the age like change in contraction of gallbladder, ability to concentrate bile are also incriminated in gallstone formation including pigment or mixed stones.

**3) Rapid weight loss**

The physiological alterations that lead to gallstone formation as a result of rapid weight loss are multiple.

i. Hepatic cholesterol secretion increase during caloric restriction.

ii. Increase secretion of mucin which is potent stimulator of cholesterol crystal formation.

iii. Decrease gall bladder motility leading to biliary sludge formation.

Gallstone formation can be prevented by administration of ursodeoxycholicacid in these patients. It is also found that there is decrease in gallstone formation in obese persons who are taking low caloric diet[60].

**4) Pathophysiology of gallstone formation in obese persons**

In obese persons hepatic cholesterol synthesis is increased and cholesterol saturation index (CSI) more. Gallbladder bile is supersaturated with cholesterol. Secretion of bile salts and phospholipids is either normal or increased. Gallbladder contractility may be decreased in the obese persons. So gallbladder stasis with supersaturated bile lead to gallstone formation.

**5) Pregnancy and Parity**

Due to increase eostrogen level bile became more lithogenic due to increase in cholesterol secretion and supersaturation of bile. Gallbladder volume will be doubled and stasis develops with formation of biliary sludge. Higher progesterone levels also impair gallbladder motility.

Both biliary sludge and stones are silent in nature but it may become symptomatic. After delivery in 60-70% pregnant woman biliary sludge disappears and gallstones disappears in 20-30%[61].

**6) Drugs**

Drugs which increases the gallstone formation are oestrogens, oral contraceptives, clofibrate, octreotide, cefriaxone (third generation cephalosporin)[62].

**7) Systemic diseases**

Gallstone formation is common in diabetic persons and its complications are also more. Insulin resistant diabetes mellitus is associated with hypertriglyceridemia, obesity, hypomotility of gallbladder leading to biliary sludge formation which inturn may lead to gallstone formation. The prevalence of gallstones in persons who had spinal cord injury is about 31% and biliary complications occur in 2.2% Hence biliary stasis is likely the cause of gallstone formation.

**8) Cirrhosis of liver**

Gallstone formation is 2-3 times greater in cirrhotic patients than a non cirrhotic population at all ages[63]. In advanced cirrhosis there is marked reduction in bile salt secretion. It is stated that decrease in bile salt is matched by diminished biliary lecithin and cholesterol and bile is not lithogenic. Gallstone in cirrhosis and other chronic liver disease is usually due to chronic haemolysis and majority of the stones are pigment type. Jaundice in cirrhosis is more likely to be due to hepatic decompensation than a stone in the CBD.

**9) Ileal disease or resection:**

In Crohn's disease with extensive involvement of ileum and major resection of ileum lead to malabsorption of bile salts. This inturn leads to increased cholesterol and supersaturated bile. Therefore gallstone formation is more. Gallstone are usually cholesterol type[64].

**10) Gastric surgery**

Gastric bypass surgery for peptic ulcers and for gross obesity is complicated with increase prevalence in gallstone formation[65]. Truncalvagotomy will adversely affect gallbladder emptying or bile lipid composition.

**11) Haemolytic anaemia**

Patients with haemolyticanaemia and hereditary spherocytosis is associated with increased incidence of pigment gallstone formation due to haemolysis[66].

**12) Other conditions**

Children with cystic fibrosis have increased incidence of gallstones. Association with peptic ulcer and hyperparathyroidism – a firm evidence is not available.

### 3.6.5 Description of Protective Factors

**1) Statins[67]**

Use of statins has been associated with a decreased risk of gallstone disease in 2 large case-control studies. The first study compared 27,035 patients with gallstone disease requiring cholecystectomy with 106,531 matched controls and showed a benefit to long-term statin use (>20 prescriptions filled and use of statins for >1.5 years); statin use was associ- ated with a decreased risk of gallstone disease requiring cholecystectomy (adjusted odds ratio [OR], 0.64). Similar results were observed in a population study from Denmark of 32,494 patients with gallstone disease matched with 324,925 controls. The odds ratio of having gallstone disease in current and prior users of statins (>20 prescriptions filled) was 0.76 and 0.79, respectively, compared with controls.

**2) Ascorbic acid[68]**

The observation that deficiency of ascorbic acid (vitamin C) is associated with development of gallstones in guinea pigs prompted investigation of the relationship between ascorbic acid levels and gallstones in humans. Serum ascorbic acid levels have been correlated with clinical or asymptomatic gallstones in 7042 women and 6088 men who were enrolled in the Third National Health and Nutrition Examination Survey. Among women, but not men, each standard deviation increase in serum ascorbic acid levels was associated with a 13% lower prevalence of clinical gallbladder disease.

**3) Coffee[69]**

In a 10-year follow up of 46,000 male health professionals, subjects who consistently drank 2 to 3 cups of regular coffee per day were approximately 40% less likely to develop symptomatic gallstones. Drinking 4 or more cups per day was even more beneficial (relative risk 0.55), but there was no benefit to drinking decaffeinated coffee. A similar benefit to regular coffee was noted in a cohort study involving 81,000 women.

## 3.7 PATHOPHYSIOLOGY OF GALL STONES

Pathogenesis of gallstone is multifactorial. There are significant difference in etiology of cholesterol and pigment gallstone. This understanding of this factor is important to prevent the disease and for treatment modalities. Gallstones are concretions and aggregations that are formed as a result of imbalance between bile acids and cholesterol in the ratio 1:10.

The Figure shows interactions of 5 primary defects that lead to formation of cholesterol gallstones[16]: (1) certain genetic factors, including LITH genes, (2) hepatic hypersecretion of biliary cholesterol, (3) gallbladder hypomotility, (4) rapid phase transitions of cholesterol, and (5) certain intestinal factors. These defects act together to facilitate cholesterol nucleation and crystallization, and ultimately promote formation of cholesterol gallstones.

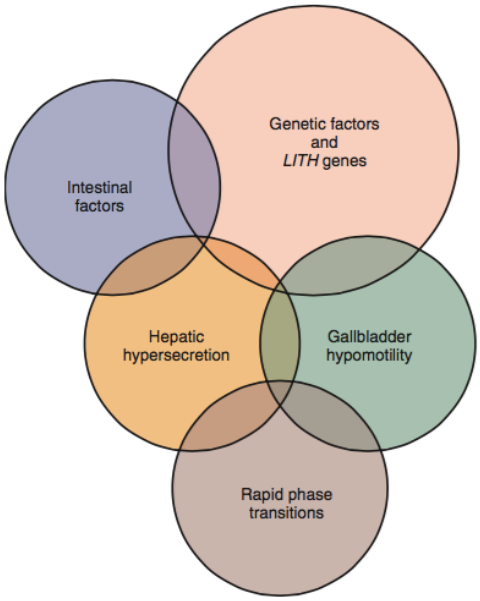



**Figure 17**. Venn diagram of 5 primary defects that work together to promote formation of cholesterol gallstones. The 5 defects are genetic factors and lith (gallstone) genes, gallbladder hypomotility, rapid phase transitions, hepatic hypersecretion of cholesterol, and intestinal factors

### 3.7.1 Stages of gallstone formation

**1) Cholesterol saturation**[70]

Cholesterol is not soluble in bile. Bile acids are amphipathic compounds with one end being hydrophilic and polar and other end being hydrophobic and nonpolar. These ionized molecules form micelles in dilute solutions with hydrophobic end inwards and hydrophilic end outwards. Incorporation of lecithin into the micelle allows H2O to penetrate the structure causing swelling. This process increase the ability of the micelle to transport greater amount of cholesterol. Recent information indicates that no more than 30% of cholesterol is transported in micelles.

The relative amounts of cholesterol transported by vesicles and micelles is related to the degree of bile saturation and crystal precipitation and stone formation. Cholesterol super saturation can occur secondary to secretion of hepatic bile with increased amounts of cholesterol or increased amounts of bile acids or lecithin.

Sequence of events in cholesterol stone synthesis includes -

**Nucleation**[71] – Aggregation of cholesterol crystals with in a supersaturated bile solution. Cholesterol monohydrate crystals form and agglomerate to become macroscopic stones. Mucin is a pronucleating factor and act as a matrix on which crystals can conglomerate and clusterise.

**Stone growth**– It is natural consequence of cholesterol precipitation and conglomeration.

**2) Gallbladder factors**

Gallbladder contributes in gallstone formation by a complex interaction of muscular and mucosal events.

**a) Stasis**[72]

Gallbladder's ability to empty is more slow and incomplete in cholelithiais. This muscle abnormality precedes gallstone formation and persists after the gallstone have been removed by dissolution therapy. This stasis is a feature of both cholesterol and pigment stones. Other factors are like sequestration of bile acids within the gallbladder reducing the amount of bile salts available for cholesterol solubalisation, alterations in the secretory or absorptive function of gallbladder leading to biliary stasis.

**b) Phospholipids in bile**[73]

Studies indicate that gallstone formation is accompanied by an increase in arachidonic acid containing phospholipids. Increased hydrolysis of arachidonyl lecithin provides the substrate for formation of prostanoids in the gallbladder wall. This activation of the prostanoid synthetic cascade is accompanied by reduced gallbladder motility and increase in mucin production by the gallbladder mucosa.

**c) Bile mucus glycoprotiens**[74]

The excessive production of glycoprotiens by gallbladder mucosa precedes stone formation. Mucin gel interferes with gallbladder contractility and emptying and acts as a nucleating matrix for cholesterol crystals to form cholesterol phospholipids vesicles.

**d) Calcium**[75]

Role of calcium is indicated by the presence of calcium salts in majority of gallstones. Preliminary results suggest that gallbladder bile from patients with cholesterol gallstones contain high levels of calcium. Mechanism by which biliary calcium increases the formation gallstones remains unknown but possible explanation includes enhanced absorption of water and solutes by the gallbladder and increase gallbladder secretion of calcium, or decrease absorption of calcium. Crystalline structures of calcium carbonate and cholesterol monohydrate crystals provide frame work for gallstone formation. In addition to the structural role, data suggests that calcium promotes fusion of vesicles and evaluates cholesterol crystal growth.

**3) Epitaxy**[76]

This is the phenomenon of the growth of one compound in one or more particular orientation on the substrate of another with near geometrical fit between respective networks which are in contact. Studies have shown that expitaxial role plays a significant part in almost all cases.

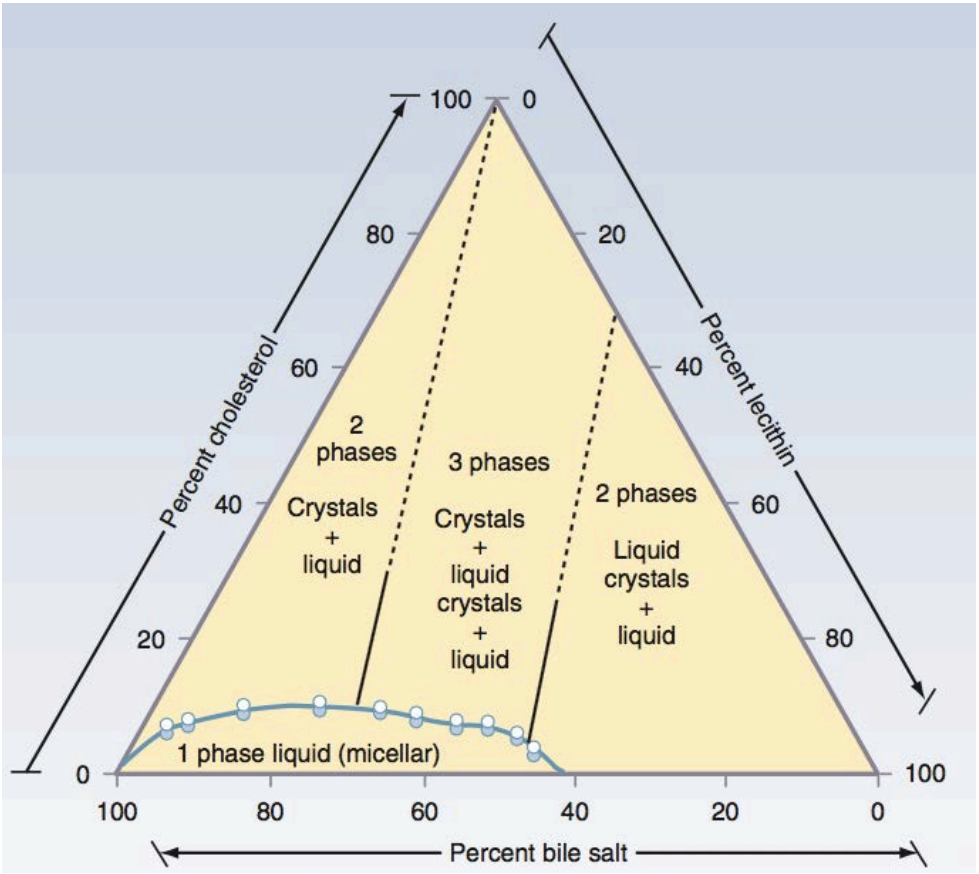
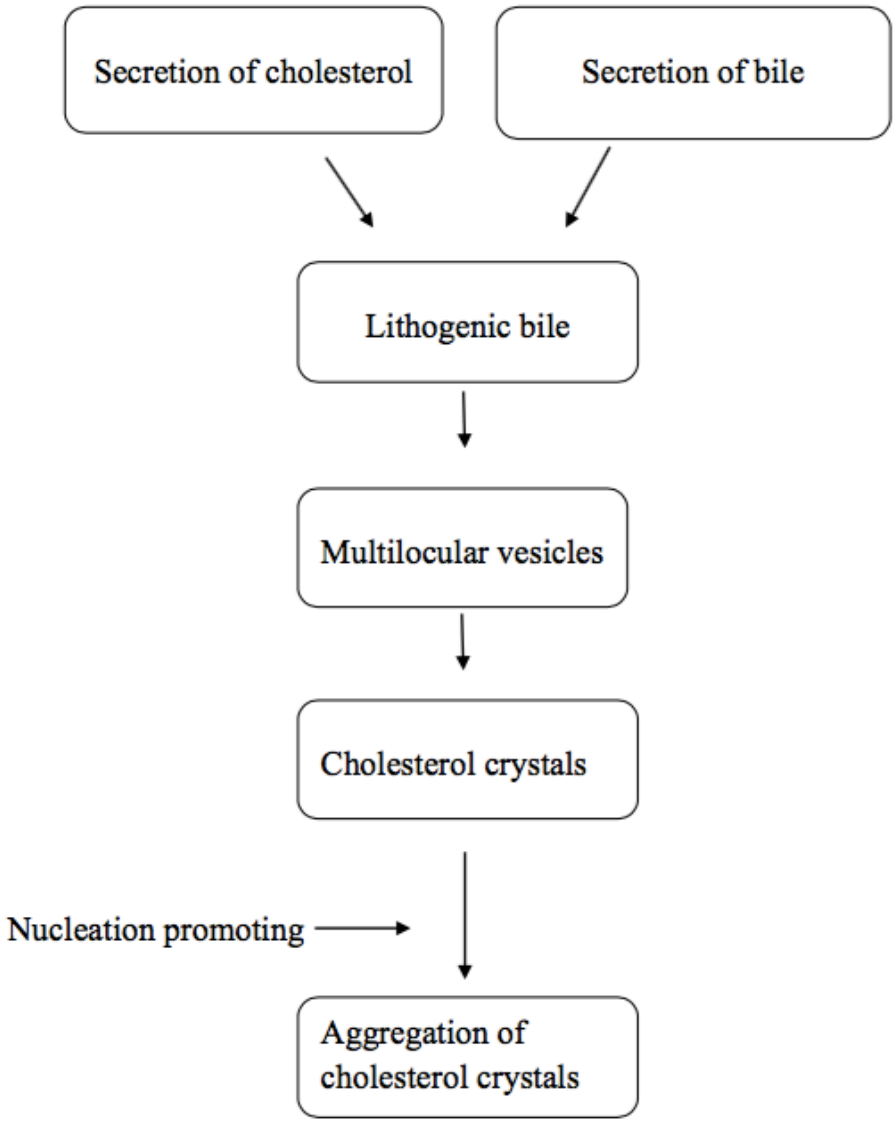



**Figure 18**:**Triangle of Solubility**.With the three major components of bile that determine cholesterol solubility and stability, each can be quantified by molar % to show a relative ratio to the other two. Cholesterol is completely soluble in only the small area in the left lower corner, where a clear micellar solution exists, below the closed circles. Just above this, in the area between the open and closed circles, cholesterol is supersaturated but stable, and thus only crystallized with stasis. in the remainder of the triangle, cholesterol is significantly supersaturated and unstable. in this region, crystals form immediately.

**Figure 19. Sequence of events in Cholesterol Lithogenesis**




### 3.7.2 Pigment gallstones:

This term is used for stones containing less than 25% cholesterol. They are irregular or smooth and amorphous or crystalline on cross-section. They represent quarter of gallstones removed at cholecystectomy. There are two types: black and brown.

Black pigment stones are largely composed of an insoluble black pigment polymer mixed with calcium phosphate and carbonate. They are usually limited to

the gallbladder. They accompany chronic haemolysis, usually hereditary spherocytosis or sickle cell disease, and mechanical prostheses in the circulation.

**Brown Pigment Stones** have calcium bilirubinate as their major component but calcium palmitate and cholesterol are other major constituents. They are usually radiolucent. They are found in the intra-hepatic and extra-hepatic bile ducts and in the gallbladder. They are virtually 10% associated with stricture, sclerosing cholangitis and Caroli's syndrome. Recurrent bile duct stones are usually of this type.In Oriental countries, they are associated with parasitic infestations of the biliary tract such as Clonorchissinensis or Ascarislumbricoides.

## 3.8 CLINICAL FEATURES

### 3.8.1 Clinical presentation of gallstones[77]:

1. Asymptomatic.
2. Biliary colic.

i. Right subcostal or epigastric pain radiating to back or lower pole of scapula lasting for 20 minutes to 6 hours.

ii. Associated with vomiting, brought on by (any) food.

iii. May disturb sleep.

3. Flatulent dyspepsia.
4. Acute Cholecystitis - Calculous (as opposed to Acalculous) / Empyema gallbladder

*Gangrenous Gallbladder:*

i. Severe pain and tenderness in right subcostal region - Murphy sign - pain on palpation of the right upper quadrant when the patient inhales.

ii. Fever and leucocytosis.

5. Chronic calculous Cholecystitis - repeated episodes of right hypochondrial pain with/without fever and vomiting.
6. Cholangitis - Fever with chills/rigors, transient jaundice, upper abdominal pain, vomiting - Charcot triad (right upper quadrant pain, fever, and jaundice)
7. Mucocele - Heaviness in upper abdomen; palpable lump.
8. Choledocholithiasis with extra-hepatic cholestasis.

9. Biliary pancreatitis.

10. Gallstone ileus.

11. Gallbladder perforation.

12. Gallbladder carcinoma.

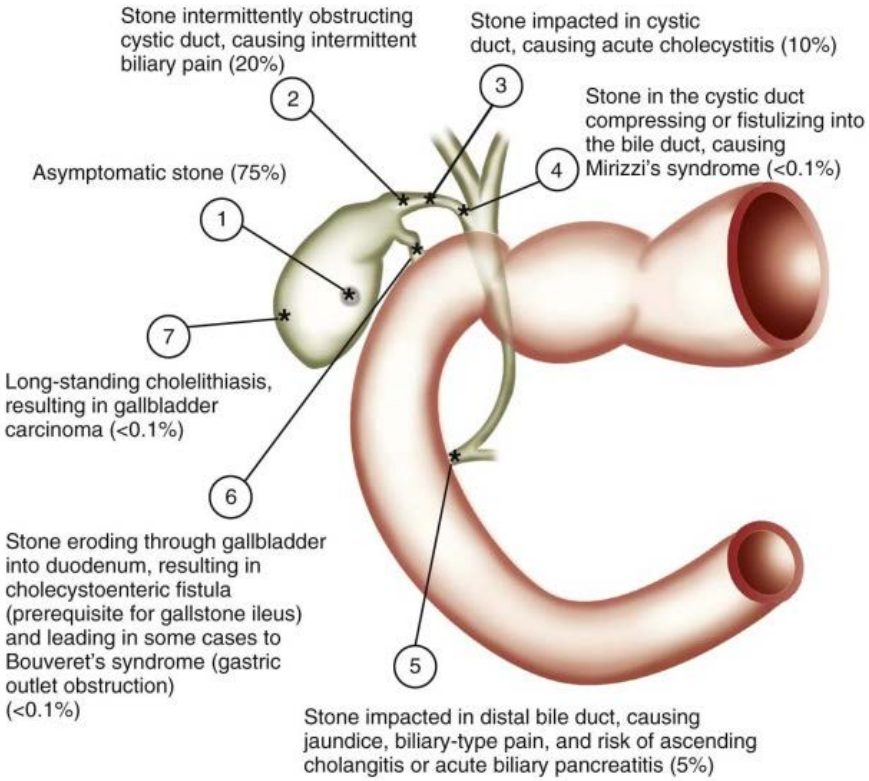



**Figure 20**: Schematic depiction of the natural history and complications of gallstones. The percentages indicate the approximate frequencies of complications that occur in untreated patients, based on natural history data. The most frequent outcome is for the patient with a stone to remain asymptomatic throughout life (1). Biliary pain (2), acute cholecystitis (3), cholangitis (5), and pancreatitis (5) are the most common complications. Mirizzi's syndrome (4), cholecystoenteric fistula (6), Bouveret's syndrome (6), and gallbladder cancer (7) are relatively rare.

### 3.8.2 Symptomatic gallstones:

**1) Chronic calculous cholecystitis**

Ongoing inflammation with recurrent episodes of biliary colic or pain from cystic duct obstruction is referred to as chronic cholecystitis. Histologically, chronic cholecystitis is characterized by an increase in subepithelial and subserosal fibrosis and a mononuclear cell infiltrate.

The primary symptom of chronic cholecystitis or symptomatic cholelithiasis is pain, often referred to as biliary colic. The pain is constant and usually lasts 1 to 5 hours. Other symptoms such as nausea and vomiting often accompany each episode, and bloating and belching may also be present in 50% of cases. Fever and jaundice are rare with simple biliary colic. During an episode of biliary colic, mild right upper quadrant tenderness may also be present.

An abdominal ultrasound is the standard diagnostic exam for gallstones. If the patient has recurrent attacks of typical biliary colic and sludge is detected on two or more occasions, cholecystectomy is indicated. In addition to sludge and stones, cholesterolosis and adenomyomatosis of the gallbladder may cause typical biliary symptoms and may be detected on ultrasonography. Cholesterolosis is caused by the accumulation of cholesterol in macrophages in the gallbladder mucosa, either locally or as polyps. It produces the classic macroscopic appearance of a “strawberry gallbladder.”

The optimal treatment for patients with symptomatic cholelithiasis is elective laparoscopic cholecystectomy. Patients should be advised to avoid dietary fats and large meals while awaiting surgery. Diabetic patients should have a

cholecystectomy promptly because they are at higher risk for acute cholecystitis or even gangrenous cholecystitis.

**2) Acute calculous cholecystitis**

Acute cholecystitis is related to gallstones in 90% to 95% of cases. Obstruction of the cystic duct leading to biliary colic is the initial event in acute cholecystitis. If the cystic duct remains obstructed, the gallbladder distends, and the gallbladder wall then becomes inflamed and edematous. In the most common scenario, the gallstone dislodges, and the inflammation will gradually resolve.

Right upper quadrant pain, similar in severity to but much longer in duration than pain from previous episodes of biliary colic, is the most common symptom of acute cholecystitis. Other common symptoms include fever, nausea, and vomiting. On physical exam, right upper quadrant tenderness and guarding are usually present inferior to the right costal margin, distinguishing the episode from simple biliary colic.

Ultrasound is the most useful radiographic test for diagnosing acute cholecystitis. It is sensitive for identifying the presence of gallstones. Biliary radionuclide scanning is used less frequently today but may be helpful in atypical cases. No filling of the gallbladder with the radiotracer (99mTc-HIDA) after 4 hours indicates an obstructed cystic duct.

After the diagnosis of acute cholecystitis is made, IV fluids, antibiotics, and analgesia should be initiated. Antibiotics should cover gram-negative aerobes as well as anaerobes. Cholecystectomy is the definitive treatment for patients with acute cholecystitis. Early cholecystectomy performed within 2 to 3 days of

presentation is preferred over interval or delayed cholecystectomy that is performed 6 to 10 weeks after initial medical therapy.

### 3) Choledocholithiasis

Common bile duct stones are classified by their point of origin and are found in 6% to 12% of patients with stones in the gallbladder. Common duct stones are also defined as retained if they are discovered within 2 years of cholecystectomy, or recurrent if they are detected more than 2 years after cholecystectomy. The secondary stones are usually of the brown pigment type. The primary stones are associated with biliary stasis and infection and are more commonly seen in Asian populations.

Common bile duct stones may be silent and are often discovered incidentally. In these patients, biliary obstruction is transient, and laboratory tests may be normal. Clinical features suspicious for biliary obstruction due to common bile duct stones include biliary colic, jaundice, lightening of the stools, and darkening of the urine. In addition, fever and chills may be present in patients with choledocholithiasis and cholangitis.

Ultrasonography, commonly the first test, can document stones in the gallbladder and estimate the diameter of the common bile duct. A dilated bile duct (>8 mm in diameter) on ultrasonography in a patient with gallstones, jaundice and biliary pain is highly suggestive of choledocholithiasis. MRC provides excellent anatomic detail, with sensitivity and specificity of 95% and 98%, respectively. ERCP is the diagnostic and potentially therapeutic test of choice for patients with suspected common bile duct stones.

The use of endoscopic cholangiography in patients with suspected common bile duct stones not only confirms the diagnosis but also provides ductal clearance of the stones and sphincterotomy before subsequent laparoscopic cholecystectomy. Prompt cholecystectomy after endoscopic clearance of the common bile duct should be performed during the hospital admission if the patient is fit for surgery.

Laparoscopic common bile duct exploration through the cystic duct or with formal choledochotomy allows the stones to be retrieved during the same procedure. If a choledochotomy is performed, a T tube is left in place. The purpose of the T tube is to provide access to the biliary system for postoperative radiologic stone extraction.

**4) Gallstone Pancreatitis**

Blockage of the pancreatic duct by an impacted stone or temporary obstruction by a stone passing through the ampulla may lead to pancreatitis by an unknown mechanism. An ERCP with sphincterotomy and stone extraction is the initial treatment and may relieve the pancreatitis. Once the pancreatitis has subsided, the gallbladder should be removed during the same admission.

## 3.9 INVESTIGATIONS

To date there are no serum or other lab tests that are absolutely specific for the presence of gallstones. In acute cholecystitis due to gallstones patient will have leucocytosis. There may be mild elevation of transaminases and alkaline phosphatase. In CBD stones serum alkaline phosphatase will be elevated along with serum gamma glutamyltranspeptidase.[78]

The following investigations can be broadly used in patients with gall stone diseases:

1. Blood investigations.
2. Plain X-ray abdomen.
3. Oral Cholecystography.
4. Cholangiography.
5. Ultrasonography (USG).
6. Computed Tomography (CT).
7. Magnetic Resonance Cholangio Pancreatography (MRCP).
8. Endoscopic Retrograde Cholangio Pancreatography (ERCP).
9. Hepatobiliary Scintigraphy.

### 3.9.1 Laboratory tests

Although termed liver function tests, the routine hepatic panel for most laboratories tests a number of aspects of metabolic and hepatic activity. The tests most useful for evaluation of biliary physiology include determination of levels of bilirubin, alkaline phosphatase, seen in any cholestatic process, and serum trans-aminases, suggesting evidence of hepatocellular injury. Bilirubin can be

subdivided into the conjugated and unconjugated forms, thereby allowing delineation of cause based on cellular location of derangement. In other words, hyperbilirubinemia may be caused by increased synthesis of bilirubin, impaired hepatocyte uptake of unconjugated bilirubin, decreased intracellular conjugation, reduced intracellular transport and excretion of conjugated bilirubin, or obstruction of the biliary tree. Although an oversimplification of a complex process, derangements up to and including conjugation will manifest as elevated unconjugated bilirubin levels[79].

### 3.9.2 Imaging modalities

**1) Abdominal x-ray**

Only 10% gallstones are radiopaque and can be visualized on a plain X-Ray of the abdomen. Thus plain radiograph is not very sensitive for gall stones.

**2) Oral cholecystography**

For years this test was the mainstay and gold standard for the diagnosis of gallstone though now it has been replaced by USG except where function of the gallbladder has to be assessed. Cholecystography is more accurate than USG in terms of quantification of the number of stones and their sizes[80]. The sensitivity for detection of radiolucent stones exceeds 90% but visualization of the ductal stones is obtained in only 20%.[81]

**3) Abdominal USG**

This is the preferred investigation for suspected cholelithiasis or cholecystitis. Examination should be performed after overnight fast of 8 to 12 hours. Two types of transducers are used, 3.5 MHZ for most of the patients. 5

MHZ provides superior imaging resolution and can be used in obese patients. Major signs of diagnosis of acute cholecystitis are demonstration of gallstones or edema or gas in the gallbladder wall. Non visualization of the gallbladder is also a major sign. Simple wall thickening is a minor sign as is local tenderness, a round shaped or dilatation. Pericholecystitic fluid is also a minor sign. The demonstration of major and minor sign together gives an overall accuracy of over 90%.When the gallbladder is normal, ultrasound often indicates other pathologies.

In chronic cholecystitis the wall is also thickened but lacks the echo poor halo and there is no local tenderness. The gallbladder fails to empty after a meal or CCK Challenge. Stones are usually present. A mucocele appears as a large, sometimes enormous gallbladder which is non tender and thin walled. The contents are usually echo free, apart from stones, though debris may form.

In general ultrasonography has distinct advantages over conventional oral cholecystography. These include absence of radiation exposure, independent of patient compliance and the lack of requirement for an intact digestive and hepatic system. In addition to identifying stones within the gallbladder or bile duct, abdominal ultrasonography provides important ancillary information regarding the anatomy of bile ducts, pancreas, and other structures in the upper abdomen. The newer techniques of sonography include the endoscopic ultrasonography. It is more sensitive in identifying small gallstones and also common bile duct stone. Endoscopic ultrasonography is useful for detecting small gallbladder stones missed on transabdominal imaging, especially those located in the neck of the

gallbladder, where duodenal gas can obscure the image when scanning percutaneously.

Sensitivity of USG to detect cholelithiasis is 95-99%. They are seen as echogenic foci with accousting shadowing and move with change in posture. This can detected the gallstones of about 1mm in size. The difficulty in USG is its limitation in measuring large gallstones and quantifying multiple gallastones.[82]

**4) CT Scan**[83]

Although ultrasound is clearly the first test of choice for delinea- tion of biliary pathology, computed tomography (CT) provides superior anatomic information. Because most gallstones are radiographicallyisodense to bile, many will be indistinguishable from bile. However, because ultrasound is operator-dependent nd provides no anatomic reconstruction of the biliary tree, CT can be used to identify the cause and site of biliary obstruction. When performed for the evaluation of hepatic or pancreatic parenchyma or possible neoplastic processes, CT is invaluable in preoperative planning, and the use of arterial phase, portal venous phase, and delayed phase imaging, known as a triple-phase CT, has essentially replaced diagnostic angiography of the liver.

**5) MRI and MRCP**

Magnetic resonance imaging (MRI) uses the water in bile to delineate the biliary tree and thus provides superior anatomic definition of the intrahepatic and extrahepatic biliary tree and pancreas. Although management of most patients with biliary pathology does not require the fine detail of anatomic evaluation shown by cross-sectional imaging, MRI is noninvasive, requires no radiation

exposure, and can prove extremely useful when planning resection of biliary or pancreatic neoplasms or management of complex biliary pathology[84]. By using the water content of bile, a cholangiopancreatogram can be created.

**6) ERCP**

Endoscopic retrograde cholangiopancreatography (ERCP) is an invasive test using endoscopy and fluoroscopy to inject contrast through the ampulla and image the biliary tree. Although it does carry a complication rate of up to 10%, its usefulness lies in its ability to diagnose and treat many diseases of the biliary tree. For patients with malignant obstruction, ERCP can be used to provide tissue samples for diagnosis while also decompressing an obstruction, but does not stage patients accurately. Many benign diseases, such as choledocholithiasis, can be easily treated by endoscopic means. ERCP has also proven extremely useful in the diagnosis and treatment of complications of biliary surgery[85].

**7) Hepatic Iminodiacetic Acid Scan**

Although incapable of providing precise anatomic delineation of pathophysiology, biliary scintigraphy, also known as a hepatic iminodiacetic acid scan (HIDA) scan, can be used to evaluate the physiologic secretion of bile. The injection of an iminodi- acetic acid, which is processed in the liver and secreted with bile, allows identification of bile flow. Therefore, the failure to fill the gallbladder 2 hours after injection demonstrates obstruction of the cystic duct, as seen in acute cholecystitis. In addition, the scan will identify obstruction of the biliary tree and bile leaks, which may be useful in the postoperative setting. HIDA scans can also be used to determine gallbladder function, because the injection of

CCK during a scan will document physiologic ejection of the gallbladder. This may be useful in patients with biliary tract pain but without stones, because some patients have pain from impaired emptying, known as biliary dyskinesia. As a nuclear medicine test, the test demonstrates physiologic flow, but does not provide fine anatomic detail, nor can it identify gallstones[86].

**8) Cholangiography:**

Routine cholangiography during laparoscopic cholecystectomy has been advocated to confirm anatomy and thus prevent ductal injury. An intra operative cholangiography provides a "road map" of entire biliary system and aids in the dissection of the function between cystic and common bile ducts which is of great value in cases where anatomic land marks are not clearly identified or where variation to the normal ductal anatomy are present[87].

## 3.10 TREATMENT[88]

There are various treatment available for treatment of gallstones. Nevertheless, cholecystectomy remains the gold standard.

**Non invasive treatment of gallstones**

1. Oral dissolution therapy
2. Extracorporeal shock wave lithotripsy.

**Minimally invasive gallbladder procedure**

1. Percutaneous cholecystostomy
2. Contact dissolution therapy
3. Percutaneous cholecystolithotomy
4. Laparoscopic cholecystectomy

**Invasive procedure**

1. Open cholecystectomy.

## LAPAROSCOPIC CHOLECYSTECTOMY

Following the advent of laparoscopic surgery, with its accompanying smaller incisions, less pain, and shorter hospitalization, surgeons have performed an increasing number of laparoscopic cholecystectomies. Acute cholecystitis carries longer operative times and a higher conversion rate to the open procedure than when laparoscopic cholecystectomy is performed in the elective setting. General anesthesia with muscle relaxation is required when performing a laparoscopic cholecystectomy,. Therefore, one contraindication to the procedure is the inability to tolerate general anesthesia. Others include end-stage liver disease with portal hypertension, precluding safe portal dissection, and coagulopathy. Because most pneumoperitoneum laparoscopy is performed using CO2 and has a number of adverse physiologic effects, severe chronic obstructive pulmonary disease, with poor ability for gas exchange, and congestive heart failure are considered relative contraindications[33].

Patient preparation, induction of anesthesia, and sterile draping are performed as for an open cholecystectomy. Although use of a urinary catheter depends on the clinical setting, an orogastric tube is standard to decompress the stomach and help with exposure of the upper abdomen. Access to the peritoneal cavity and creation of pneumoperitoneum can be performed by the open or closed technique according to the expertise and discretion of the surgeon. The open technique involves making a small incision at the umbilicus, cutting down through the fascia of the abdominal wall, incising the peritoneum directly, and inserting a blunt trocar, known as a Hasson cannula. Alternatively, in the closed technique,

an incision is made and a needle inserted into the peritoneal cavity to insufflate the abdomen prior to the placement of any trocars. Following the establishment of a CO2 pneumoperitoneum, a brief exploration is performed and additional 5-mm ports are placed in the right anterior axillary line, right midclavicular line, and subxiphoid location. The lateral port at the anterior axillary line is used to elevate the fundus of the gallbladder toward the right shoulder. This retraction provides exposure to the infundibulum and porta hepatis. The midclavicular trocar is used to grasp the gallbladder infundibulum, retracting it inferolaterally to open the triangle of Calot By distracting Hartmann's pouch laterally, the cystic duct no longer lies almost parallel to the common hepatic duct.

The dissection is then carried along the infundibulum on the anterior and posterior surfaces to expose the base of the gallbladder. This dissection will eventually clear all fibrofatty tissue from the triangle of Calot. Inferolateral traction of the infundibulum then allows documentation of two structures entering the gallbladder, the cystic duct and cystic artery. A useful landmark for the cystic artery is the overlying lymph node, known as Calot's node. The view of the liver bed through the space between cystic duct and cystic artery and above the cystic artery is known as the critical view of safety, and minimizes the risk of inadvertent iatrogenic bile duct injury. With sufficient dissection, clips are placed on the cystic artery and cystic duct. If a cholangiography is performed, the cystic duct is only clipped adjacent to the gallbladder and the cystic duct incised, although not transected. A cholangiographic catheter is then fed through the incised duct and fluoroscopic images obtained with injection of contrast into the

cystic duct and biliary tree. On obtaining a normal cholangiogram or when cholangiography is not performed, the cystic duct is doubly clipped on the common duct side and transected. The previously clipped artery is also transected and the gallbladder dissected off the liver bed using electrocautery. Because the venous drainage of the gallbladder is directly into the liver bed through venules, excellent hemostasis must be achieved during this dissection. The cystic duct and cystic artery clips are inspected just prior to completion of the dissection of the fundic attachments, because the superior traction of the fundus has provided exposure to the porta and triangle of Calot. The gallbladder is then brought out of the abdominal cavity through the umbilical port. In the setting of acute cholecystitis, or if during dissection the gallbladder was entered, a plastic bag should be used for retrieval. Any stones that are spilled during a cholecystectomy should also be retrieved.

Opinion is sharply divided regarding the performance of selective versus routine cholangiography, with supportive data for each approach. Routine cholangiography will identify unsuspected stones in less than 10% of patients and the natural history of these asymptomatic stones suggests that they will remain asymptomatic. Iatrogenic bile duct injury occurs less often when cholangiography is performed routinely. However, even when performed routinely, cholangiograms are frequently misinterpreted and thus do not adequately prevent an injury. In many cases of laparoscopic cholecystectomy performed for biliary colic, a cholangiogram will not alter management. Also, it increases the operative time and adds fluoroscopic exposure. Indications for cholangiography in the

selective setting include unexplained pain at the time of cholecystectomy, any suspicion of current or previous choledocholithiasis without pre-operative duct clearance, any question of anatomic delineation during cholecystectomy, elevated preoperative liver enzyme levels, dilated common bile duct in preoperative imaging, and suspicion of intraoperative biliary injury.

Although just as accurate as cholangiography for the identification of choledocholithiasis, laparoscopic ultrasonography is highly operator- dependent, requires additional instrumentation, and is not widely available.

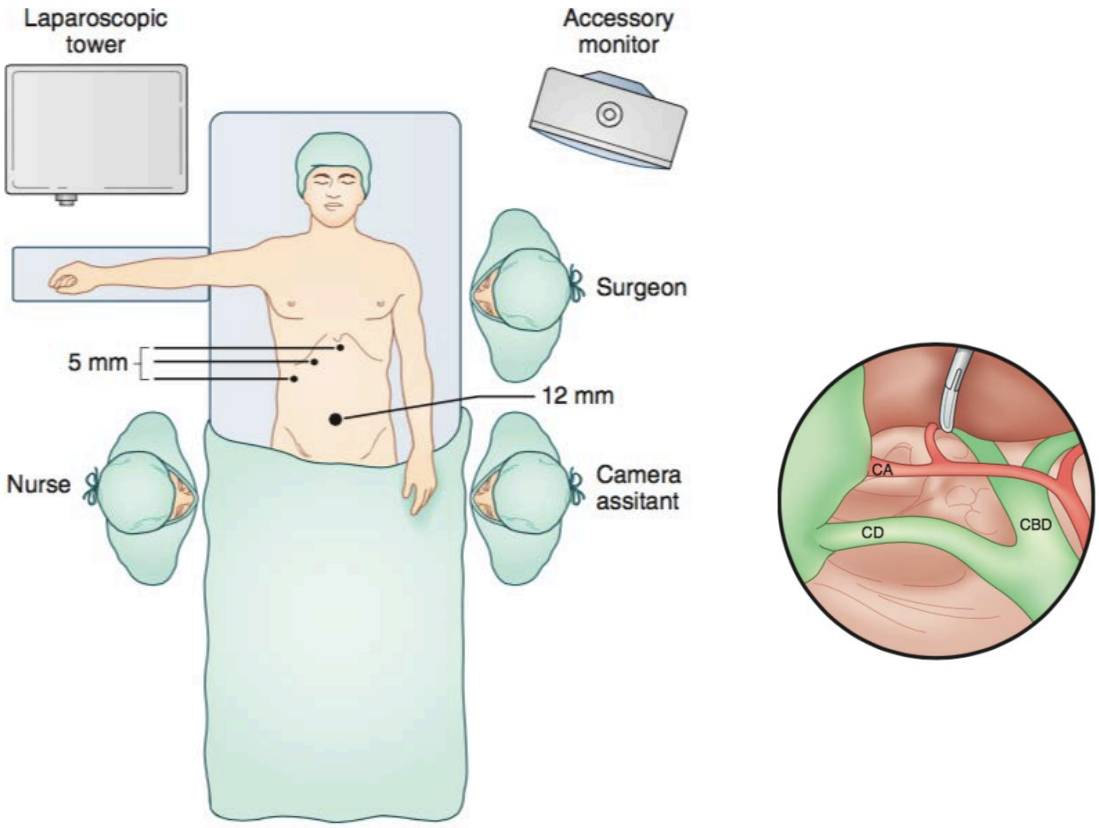



**Figure 21**. Laparoscopic cholecystectomy ports. the assistant uses the periumbilical port to provide access for the camera and the most lateral port to elevate the fundus and expose the neck. the surgeon can then provide inferolateral traction on the infundibulum and open the critical view of safety.

## OPEN CHOLECYSTECTOMY

As laparoscopic cholecystectomy has become the procedure of choice for the treatment of most gallbladder disease, experience with open cholecystectomy has drastically declined. Open cholecystectomy is generally performed following conversion from the laparoscopic approach or as a step during another operation, such as a pancreaticoduodenectomy. The open cholecystectomy can be performed through a midline or right subcostal incision. Retraction of segment IV provides exposure of the cystic duct and artery. With similar inferolateral traction to the gallbladder infundibulum, the cystic duct is taken out of alignment from the common duct for its identification and division. Early identification and ligation of the cystic artery limits the blood loss during the procedure, but may prove difficult because of inflammation.

Another approach to the gallbladder infundibulum involves dissecting the fundus off the liver in a dome- down approach. Here, the attachments of the gallbladder are divided, allowing inferolateral traction of the entire gallbladder to open the triangle of Calot and identify the appropriate duct and artery. When performed for severe cholecystitis, the dissection of the gallbladder of the liver bed may be associated with sub- stantial blood loss, but with removal of the infected gallbladder and packing of the area, the bleeding is usually well controlled.

# MATERIALS AND METHODS

# MATERIALS AND METHODS

50 patients suffering from Cholelithiasis, confirmed by USG, were divided into two groups based on serum iron values. Group A, consists of patients with normal serum iron (non-anaemic) and group B, of patients with less than normal serum iron (anaemic). Gall bladder Bile cholesterol and serum cholesterol of both the groups are compared.

TYPE OF STUDY : Cross Sectional Study

SAMPLE SIZE : 50 patients

PERIOD OF STUDY : Dec 2014 - Sep 2015

PLACE OF STUDY :

Department of General Surgery at Government Royapettah Hospital, Kilpauk Medical College Hospital, Chennai.

## 4.1 MATERIALS AND METHODS:

The study was conducted over a period of 10 months, from December 2014- September 2015. The study protocol was approved by the ethical committee of our institute. It was a retrospective analysis. The patients were selected, based only on the USG confirmation of their gall stones, irrespective of their age, sex, physique, parity, etc. Only those patients were included, whose serum as well as bile could be procured for analysis. Patients with empyema and mucocele of gall bladder were excluded.

All the patients, who were included in the study were given a serial number 1 to 50, in the order of their admission to the surgery department for Cholecystectomy. Thus their bile and serum samples were also labeled 1 to 50

accordingly. The numbered samples were sent to the Biochemistry department for analysis. All the numbered samples with less than normal serum iron (n=23) were put in the anaemic group, B and all the samples with normal serum iron (n=27) were put in the non anaemic group, Group A.

Serum iron was estimated by Ferrozine kit method for determination of iron. The normal reference values supplied with the kit, for males (60-160 μg/dl) and for females (35-145 g/dl), were used to label the patients as anaemic and non-anaemic i.e. males with serum iron < 60 g/dl and females with serum iron <35 μg/dl were labeled as anaemic. During the operation for open cholecystectomy, bile was aspirated with an aspiration needle mounted on a sterilized syringe. The aspiration needle was passed obliquely into the fundus of gall bladder and as much of bile as possible, was withdrawn from the gall bladder. Similarly during laparoscopic cholecystectomy, bile was aspirated under vision through a long venflon needle or a veress needle just before delivering out the gallbladder at the port site. Bile was kept in a sterile labeled container and sent for analysis. Serum cholesterol and gall bladder bile cholesterol of all the patients were estimated. Bile was first subjected to the Folch method to extract lipids and then the cholesterol contents were estimated as for serum cholesterol. In the Folch method, lipids from bile were extracted by using water, Methanol and Chloroform mixture in the ratio of 3:4:8 v/v and from the extracted lipids, cholesterol was estimated by Enzopak kit, based on the cholesterol oxidase/peroxidase method. The enzymes used only the cholesterol as substrate and hence Bilirubin is automatically eliminated, from the procedure of cholesterol estimation.

## 4.2 ANALYSIS:

- The biliary cholesterol levels in the 2 groups of patients A and B were compared using a student t-test to detect any statistically significant difference.
- Similarly, the serum cholesterol levels of these 2 groups were analyzed with a student t-test.
- Also, the age, sex and parity distributions of serum iron and biliary cholesterol in the study population was analyzed.

# OBSERVATON AND RESULTS

# RESULTS

During the 10 month period from Dec 2014 to September 2015, a comparative study of Biliary Cholesterol levels was done between 2 groups, divided based on Serum Iron levels among 50 consecutive patients admitted to the Department of Surgery, Govt. Royapettah Hospital, Kilpauk Medical College, Chennai with a diagnosis of Symptomatic Gallstone Disease who underwent Cholecystectomy. The results are as follows:

- Out of the 50 Patients, 40 (80%) were female and 10 (20%) were males.
- The Male to Female Ratio was 1:4.
- In the present study the minimum age was 17 yrs and the maximum age was 68 yrs.
- The number of patients was highest in the age group of 40-50 yrs having 14 patients (28%) followed by 30-40 yrs having 13 patients (26%). The least was in the age group 60-70 and 0-20 yrs having 2 patients each (4%).
- Mean age was 40.6 yrs. Standard Deviation 12.1 yrs
- Median Age was 39.5 yrs.
- The Majority of Patients Presented with all the 3 symptoms of cholelithiasis—31(62%) of the 50.

**Table 1. Age Distribution of the Study Group:**

| Age Range | Frequency | Percentage |
|---|---|---|
| <20 yrs | 2 | 4% |
| 20-30 yrs | 11 | 22% |
| 30-40 yrs | 13 | 26% |
| 40-50 yrs | 14 | 28% |
| 50-60 yrs | 8 | 16% |
| 60-70 yrs | 2 | 4% |
| **Total** | 50 | 100% |

**Chart 1. Age Distribution of Study Population**

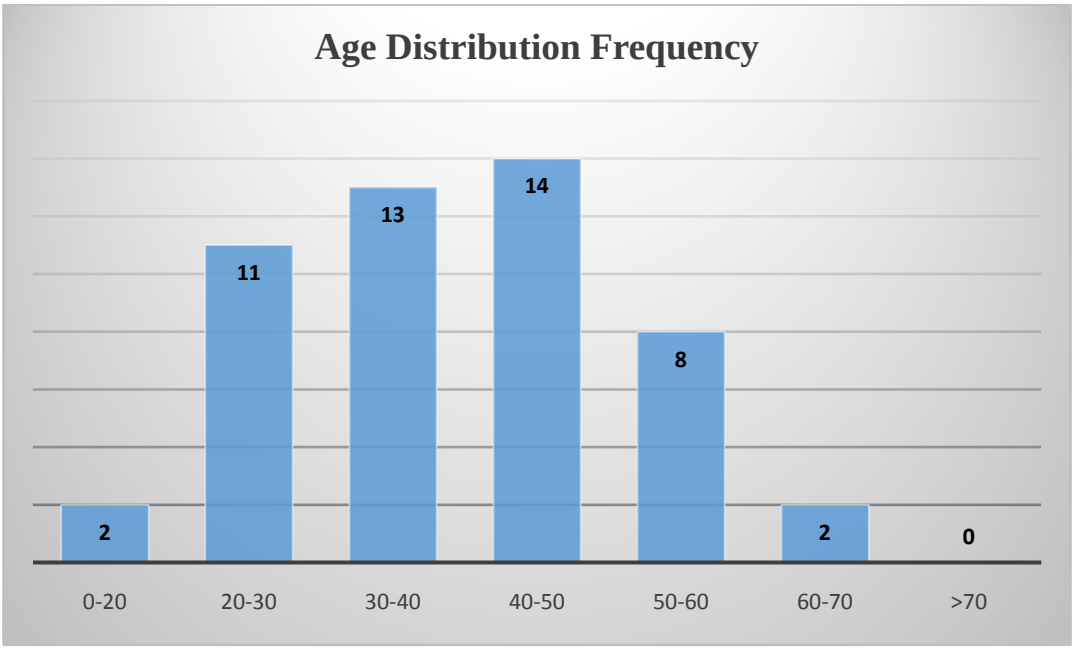


**Table 2. Sex Ratio of the Study Group:**

| Male | Female |
|---|---|
| 10 | 40 |

**Chart 2. Sex Ratio of Study Group**

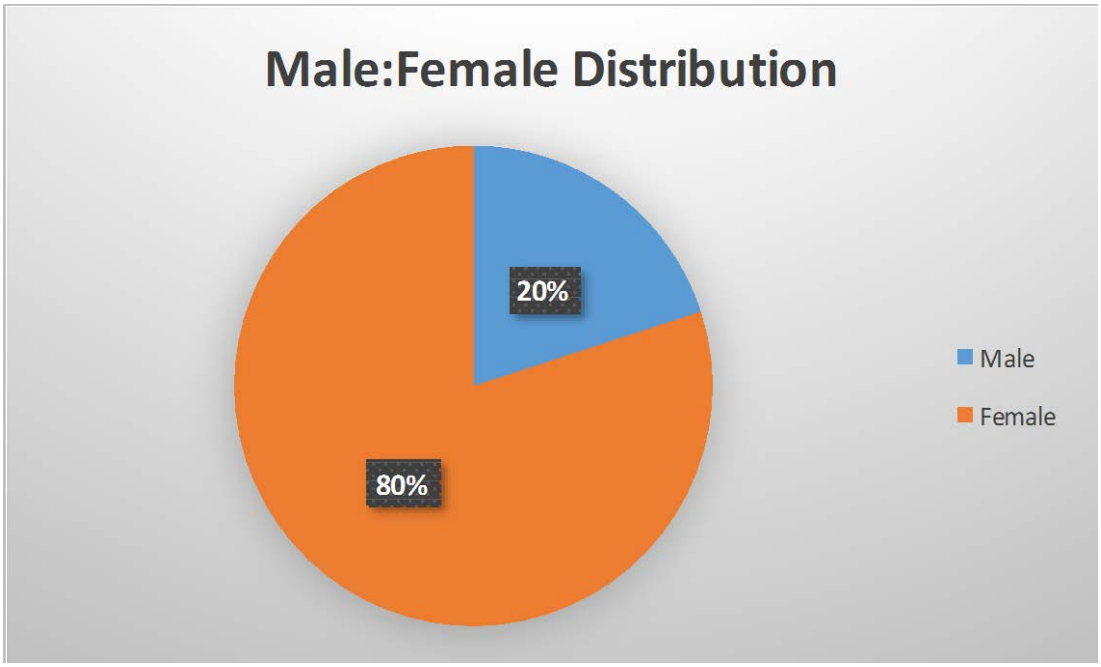
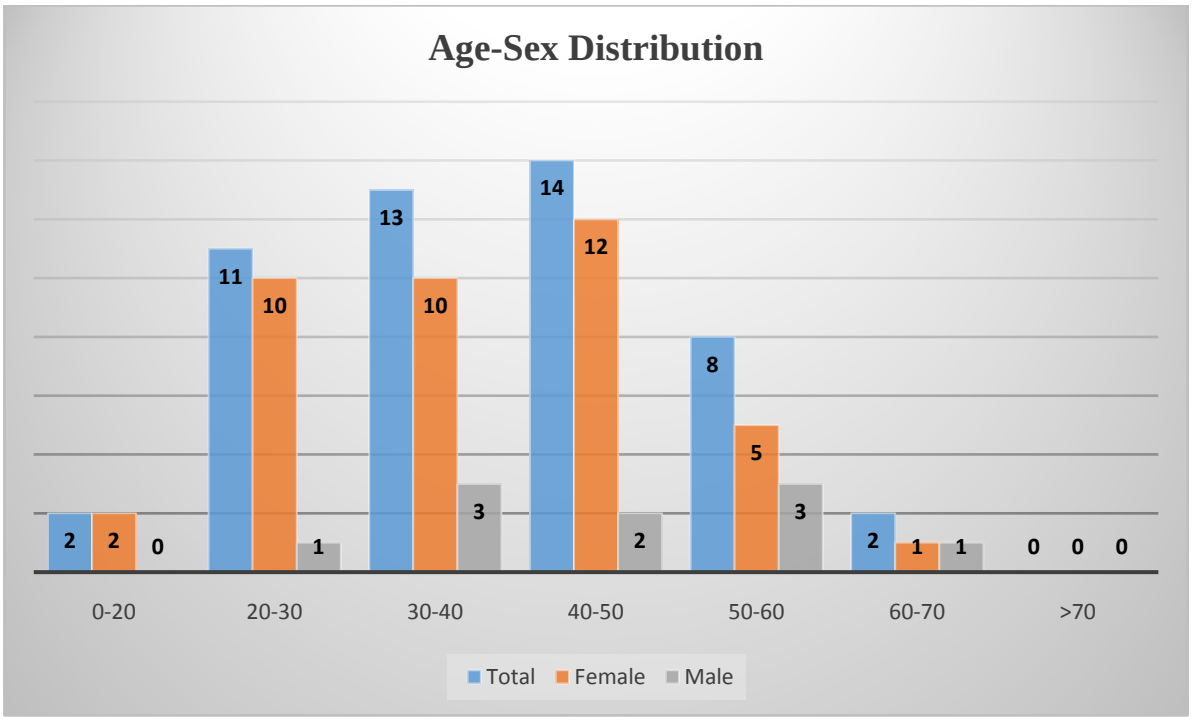


**Table 3. Combined Age and Sex distributions:**

| Age Range | Total | Females | Males |
|---|---|---|---|
| <20 yrs | 2 | 2 | 0 |
| 20-30 yrs | 11 | 10 | 1 |
| 30-40 yrs | 13 | 10 | 3 |
| 40-50 yrs | 14 | 12 | 2 |
| 50-60 yrs | 8 | 5 | 3 |
| 60-70 yrs | 2 | 1 | 1 |
| >70 yrs | 0 | 0 | 0 |

**Chart 3. Combined Age and Sex Distributions:**



***Results of the Analytical Study:***

- The 50 Patients, who were Gall Stone Sufferers were divided into 2 Groups based on the serum Iron levels.

  **Group A**: Patients with normal serum iron.

  (M: 60-160 μg /dl  F: 35-145 μg /dl)

  **Group B**: Patients with less than normal serum iron.

- 27 (54%) of them were in the Normal Iron Deficient Group (A) and 23 (46%) were in the Iron Deficient Group (B).

- **The Non Iron-Deficient patients (n=27)-Group A:**

  *The average serum iron was 93.2± 35 micro gm/dl.

  * The Mean Biliary Cholesterol Level was

  MEAN=754.5 mg/dl  SD=398.3 mg/dl

  *The Mean Serum Cholesterol Levels were 184.8 ±35 mg%

- **The Iron-Deficient patients (n=23)-Group B:**

*The average serum iron was 26.8± 8.6 micro gm/dl.

* The Mean Biliary Cholesterol Level was

MEAN=1184.7 mg/dl  SD=405.2 mg/dl

* The Mean Serum Cholesterol Levels were 172±49 mg%

**Table 4.**

**Distribution of patients into Group A and Group B:**

| Group A<br><br>(Non-Iron Deficient) | Group B<br><br>(Iron Deficient) |
|---|---|
| 27 | 23 |

**Chart 4. Distribution of patients into Group A and Group B:**

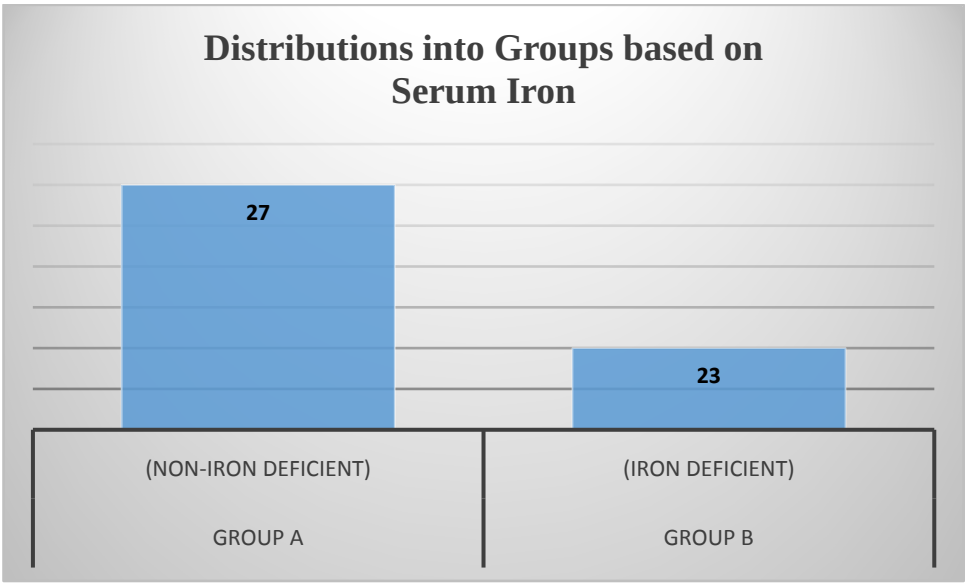


**Table 5. Serum Iron Levels in Study Group Patients:**

| Patients | No. | % | Range of Serum Iron (µg/dl) | Mean ±S.D |
|---|---|---|---|---|
| Males | | | | |
| • Non-Iron Deficient | 9 | 90 | 95.5-163 | 119.8 ± 25.3 |
| • Iron Deficient | 1 | 10 | 40 | - |
| • Total | 10 | 100 | 95.5-163 | 111.8 ± 34.7 |
| Females | | | | |
| • Non-Iron Deficient | 18 | 45 | 36.3-140.1 | 79.8 ± 31.1 |
| • Iron Deficient | 22 | 55 | 7.4-34.6 | 26.2 ± 8.3 |
| • Total | 40 | 100 | 7.4-140.1 | 50.4 ± 34.5 |

**Chart 5. Sex Distribution among Groups**

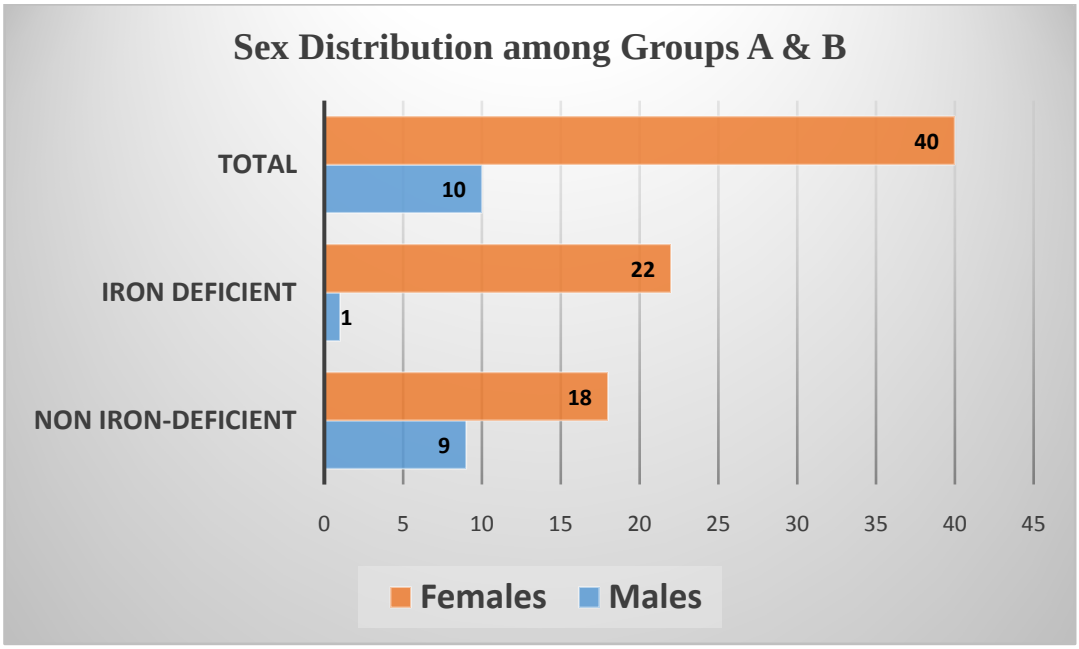


**Chart 6. Group A & B Distribution among Males and Females**

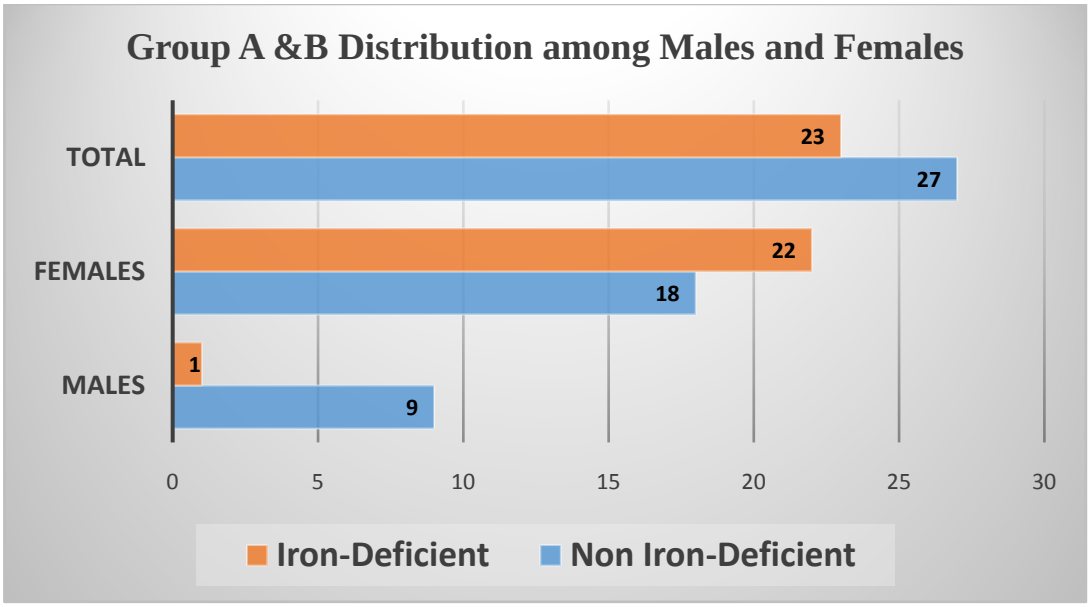


**Table 6. Serum Iron Contents in Group A & Group B Patients**

| Group | No. of Patients | Serum Iron Range µg/dl | Serum Iron Mean ±S.D |
|---|---|---|---|
| **A (Non-Iron Deficient)** | 27 | 36-163 | 93.2 ± 34.6 |
| **B (Iron deficient)** | 23 | 7-40 | 26.8 ± 8.6 |
| ***P-Value*** | | | <0.0001 |

**Chart 7. Mean Serum Iron Contents + Standard Deviation in Group A & Group B Patients**

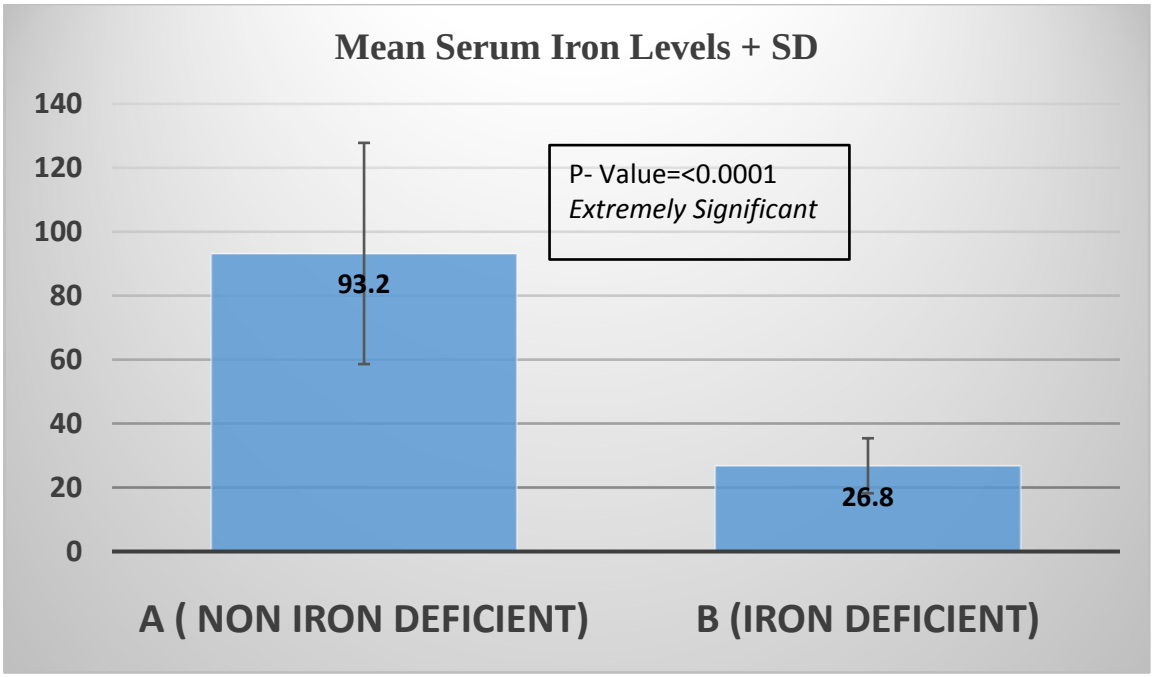


**Table 7. Biliary Cholesterol Levels in Group A & Group B Patients**

| Group | No. of Patients | Biliary Cholesterol Range mg/dl | Biliary Cholesterol Mean ±S.D |
|---|---|---|---|
| **A (Non-Iron Deficient)** | 27 | 3-147 | 754.5 ± 398.3 |
| **B (Iron deficient)** | 23 | 26-181 | 1184.7 ± 405.2 |
| ***P-Value*** | | | <0.0004 |

**Chart 8. Mean Biliary Cholesterol Levels + Standard Deviation in Group A & Group B**

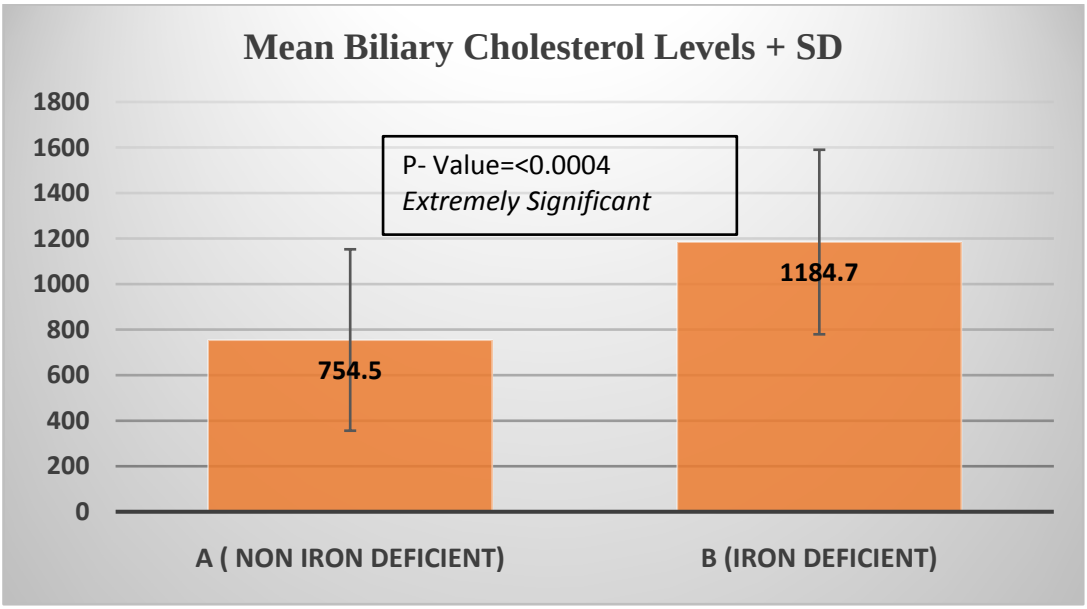


**Table 8. Serum Cholesterol Levels in Group A & Group B Patients**

| Group | No. of Patients | Serum Cholesterol Range mg/dl | Serum Cholesterol Mean ±S.D |
|---|---|---|---|
| **A (Non-Iron Deficient)** | 27 | 93-254 | 184.8 ± 35 |
| **B (Iron deficient)** | 23 | 79-270 | 171 ± 49.3 |
| ***P-Value*** | | | 0.2544 |

**Chart 9. Mean Serum Cholesterol Levels + Standard Deviation in Group A & Group B**

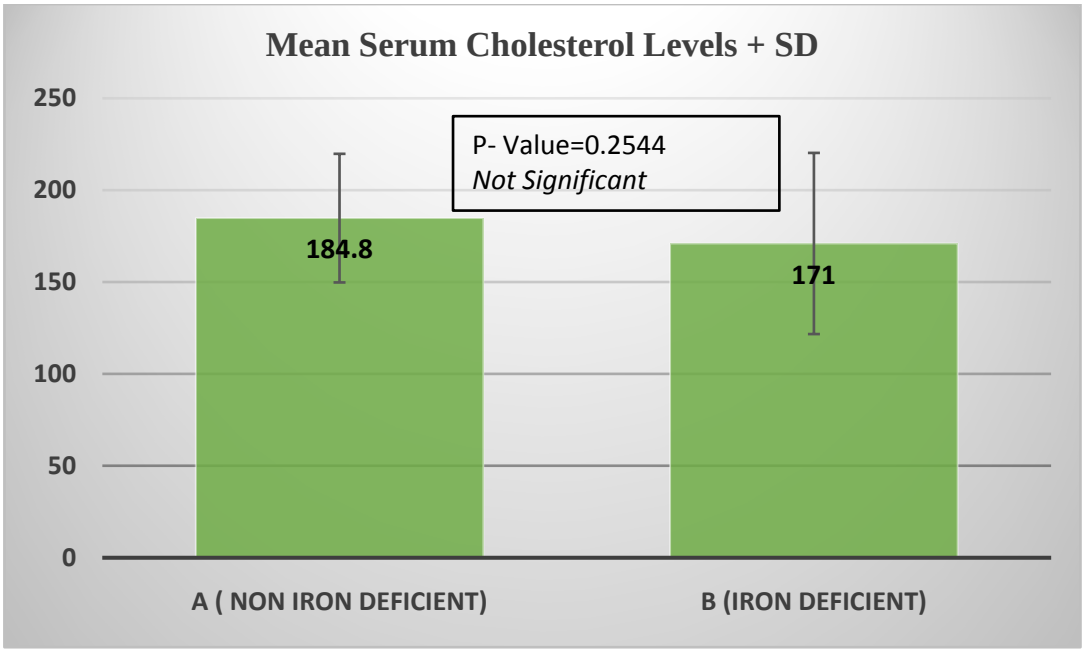


**Table 9. Serum Iron, Biliary Cholesterol and Serum Cholesterol Levels with P- Values after T- Test Analysis**

| **Groups** | **Serum Iron** (μg/dl) | **Biliary Cholesterol** (mg/dl) | **Serum Cholesterol** (mg/dl) |
|---|---|---|---|
| **A=27 (Non- Iron Deficient)** | 93.2 ± 34.6 | 754.5 ± 398.3 | 184.8 ± 35 |
| **B=23 (Iron Deficient)** | 26.8 ± 8.6 | 1184.7 ± 405.2 | 171 ± 49.3 |
| ***P-Value*** | <0.0001 ***Extremely Significant*** | <0.0004 ***Extremely Significant*** | 0.2544 ***Not Significant*** |

**INDIVIDUAL UNPAIRED T- TEST ANALYSIS:**

- **BILIARY CHOLESTEROL LEVELS:**

| Group | Group A (Non- Iron Deficient) | Group B (Iron Deficient) |
|---|---|---|
| ***Mean Biliary Cholesterol*** | 754.500 | 1184.700 |
| ***SD*** | 398.300 | 405.200 |
| ***SEM*** | 76.653 | 84.490 |
| ***N*** | 27 | 23 |

- **P value and statistical significance:**

  The two-tailed P value equals ***0.0004***

  By conventional criteria, this difference is considered to be ***extremely statistically significant.***

- **Confidence interval:**

  The mean of Group One minus Group Two equals -430.200

  95% confidence interval of this difference: From -659.252 to -201.148

- **Intermediate values used in calculations:**

  t = 3.7763

  df = 48

  standard error of difference = 113.920

- **SERUM CHOLESTEROL LEVELS:**

| Group | Group A (Non- Iron Deficient) | Group Two (Iron Deficient) |
|---|---|---|
| ***Mean Serum Cholesterol*** | 184.800 | 171.000 |
| ***SD*** | 35.000 | 49.300 |
| ***SEM*** | 6.736 | 10.280 |
| ***N*** | 27 | 23 |

- **P value and statistical significance:**

  The two-tailed P value equals 0.2544

  By conventional criteria, this difference is considered to be not statistically significant.

- **Confidence interval:**

  The mean of A (Non-Iron Deficient) minus B (Iron Deficient) equals 13.800

  95% confidence interval of this difference: From -10.254 to 37.854

- **Intermediate values used in calculations:**

  t = 1.1535

  df = 48

  standard error of difference = 11.963

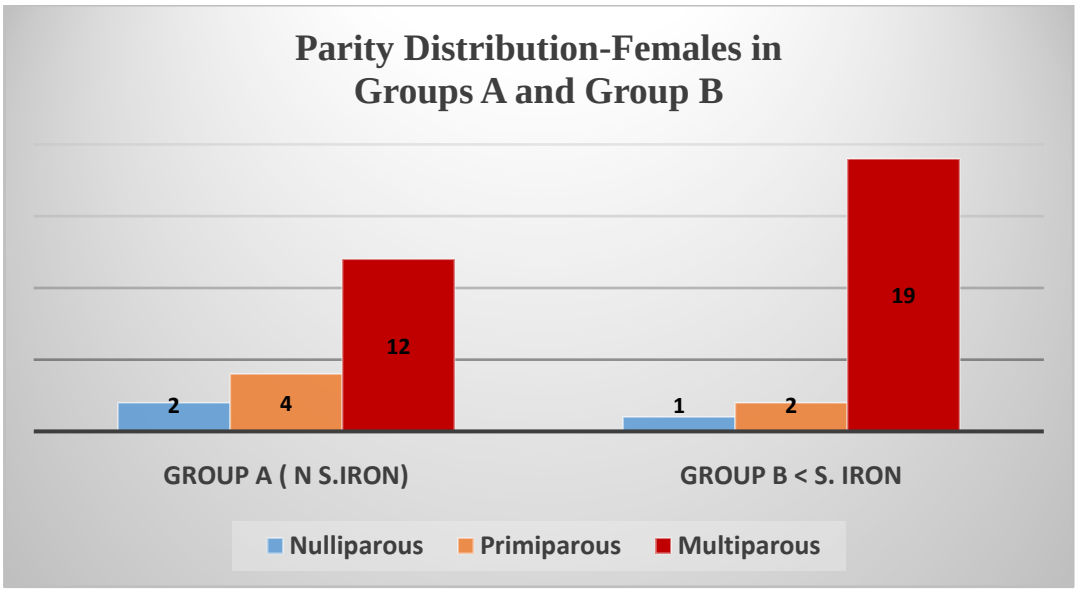
**PARITY SPECIFIC DISTRIBUTION:**

- All the female patients were subdivided based on their parity status.
- 31 (77.5) of the 40 female patients were multiparous.
- The Multiparous Females in Groups A and B were 12 and 19 respectively.
- The Primiparous Females in Groups A and B were 4 and 2 respectively.
- The Nulliparous Females in Groups A and B were 2 and 1 respectively.
- This show that multiparous females predominant in the iron deficient group.

**Table 10. Parity specific distribution of Serum Iron Levels in Female Patient**

| **Parity** | | **Group A** | ***Non Iron-Deficient*** | | **Group B** | ***Iron Deficient*** |
|---|---|---|---|---|---|---|
| | **No of Pts** | **%** | **Mean Serum Iron** | **No. of Pts** | **%** | **Mean Serum Iron** |
| **Nulliparous** | 2 | 11 | 120.6 ± 27.5 | 1 | 4.5 | 34 |
| **Primiparous** | 4 | 22 | 89.3 ± 25.2 | 2 | 9 | 33 ± 1 |
| **Multiparous** | 12 | 67 | 69.9 ± 28.5 | 19 | 86.5 | 25.1 ± 8.4 |
| ***Total*** | 18 | 100 | 79.8 ± 31.1 | 22 | 100 | 26.2 ± 8.3 |

**Chart 11. Parity specific distribution of Serum Iron Levels in Female Patient**



# DISCUSSION

# DISCUSSION

Cholelithiasis is a very common condition encountered in surgical practice. Though most cases may be asymptomatic, a definitive percentage of these patients may develop symptoms of Gall Stone disease. A few will develop complications that may even be life threatening.

Over the past century the mortality and morbidity related to Gall Stone Disease has decreased due to the early recognition and treatment of symptomatic cholelithiasis. By and large, surgical management has been the treatment of choice, and with the advent of laparoscopy, Laparoscopic cholecystectomy has replaced Open Cholecystectomy as the Gold Standard in the surgical management of uncomplicated gall stone disease.

There have been numerous studies done to assess the risk factors for Cholelithiasis. These have been discussed earlier in this study. Also its of paramount importance to identify easily modifiable risk factors as these can help in risk stratification and also prevention.

The exact mechanism of Gall Stone formation is a complex process that is not solely dependent on any single factor. Thus Gall stone disease has a multi factorial etiology. Newer risk factors/ etiological agents have been studied time and again, with some showing to have a significant impact on the disease process.

As stated already, animal studies had suggested that Iron may have a role to play in the pathophysiology of gall stone disease. More importantly the finding that iron deficient mammals were more prone to develop gall stones, has sparked interest in its probable role in humans. Also the classical teaching is that a

females, in her forties, obese and multiparous is more prone for gall stone disease. When this is viewed, keeping the recent findings in perspective, it may suggest that apart from the other risk factors in this group, these are also the patients who are most iron deficient. Hence, it makes perfect sense, more so in the Indian population, to define the role of Iron or its deficiency in the pathophysiology of gall stone disease.

As data from other studies suggest, Iron deficiency changes the activity of many liver enzymes. This may be a factor in promoting cholesterol supersaturation and increased cholesterol crystal nucleation. Also Iron acts as a coenzyme for nitric oxide synthase. Nitric oxide helps in the normal gall bladder tone and relaxation. An alteration in this fine balance may promote gallbladder stasis which results in cholesterol stone formation.

The current study was done in a randomly selected South Indian Population who presented to our hospital with symptomatic gall stone disease.

***Demographic Distributions:***

✓ ***SEX:***

- Of the 50 patients with gall stone disease, 40 were females (80%) and 10 were males(20%). This is consistent with the known sex prevalence, that gall stone disease is more common in females than males.
- The main sufferers of gall stone disease in our study were females as compared to males. The female to male ratio was 4:1. This was similar to that observed in his study by Zuhair R, Ganey et al.[89] and Major Alok Sharma et al, series . In contrast, this was higher than that observed by

Frazee et al[90],U.Berggren et al[91] and the Battacharyaseries.This is higher than that reported by most western studies indicate the female to male ratio as between 3:1 to 2:1.

- The reason for high incidence in females could be that pregnancy and child birth have a direct influence on biliary tract disease, acting by bile stasis , weight gain and consequently hypercholesteremia.

✓ ***AGE:***

- The maximum number of patients-14 (28%) of the 50- were in the age group of 40-50 yrs followed by 30-40 yrs having 13 patients (26%). The Mean age was 40.6 yrs SD-12.1 yrs.
- Similar incidence is seen in the studies of Herman et al and Hanif series showed peek incidence in 5th decade.
- In western studies the peak incidence is in the 5th and 6th decades. Similar findings are noted in the studies of Ganey et al[89] and Moreaux et al[92].

✓ ***GROUP DIVISIONS:***

- The 50 patients were divided based on their serum iron levels into 2 groups.

  ***Group A- Non Iron Deficient***

  ***Group B- Iron Deficient***

- In both the groups, females predominated more than males.

Group A= M:F=1:2

Group B=M:F=1:22

- Female patients far exceeded males in the iron deficient group (Group B), consistent with the fact that iron deficiency is more prevalent in females.

✓ ***PARITY DISTRIBUTION:***

- The 40 females, both Group A (18) and B (22) were divided based on parity. In both the groups A and B the number of multiparous females [n(A)=12,n(B)=19] who suffered from cholelithiasis was more than primipara [n(A)=4,n(B)=2] and nullipara [n(A)=2, n(B)=1]

**CRITICAL REVIEW OF TEST STATISTICS*:***

* The non-Iron deficient group (Group A) had an above average value of 93.2 ± 34.6 micro gm/dl as compared to the Iron deficient group (Group B) which had a value of 26.8 ± 8.6 micro gm/dl.

* The **Biliary Cholesterol values** for Group A and B respectively were 754.5 ± 398.3 and 1184.7 ± 405.2 mg/dl.

* The **Serum Cholesterol values** for Group A and B respectively were 184.8 ± 35 and 171 ± 49.3 mg/dl.

✓ In our study the Biliary Cholesterol levels were significantly higher in the Iron Deficient group (Group B) than compared to the Non-Iron Deficient Group. This result was extremely statistically significant with a p value of <0.0004.

✓ Also, there was no significant difference between the values of serum cholesterol levels in the Iron Deficient (Group B) and Non-Iron Deficient Group. (Group A). P value of 0.2544.

The current study suggests that deficiency in serum iron could play a role in the increased saturation of biliary cholesterol. Biliary cholesterol supersaturation is an independent factor in the formation of cholesterol gall stones. As mentioned previously, the probable explanation for this is the defective cholesterol metabolism and gall bladder stasis promoted a deficiency in serum iron.

# CONCLUSION

# CONCLUSION

- To conclude, there was a **significant difference** in the Biliary Cholesterol Levels for Non-Iron Deficient (M=754.5 mg/dl , SD=398.3) and Iron Deficient Subjects(M=1184.7 mg/dl, SD=405.2) t=3.77, p =<0.0004.

- Similarly, an independent t-test comparing Serum Cholesterol levels in Non-Iron Deficient (184.8 ± 35 mg/dl) and Iron Deficient subjects (171 ± 49.3 mg/dl) did not find a statistically significant difference. (p=0.2544)

These results suggest that Iron Deficiency has an association with Biliary Cholesterol Values.

# SUMMARY

# SUMMARY

1. The Study was done on 50 consecutive patients with Gall Stone disease who were operated in our department.
2. In this study, 40 patients (80%) were female and 10 patients (10%) were male.
3. In this study, maximum patients were from age group 40 – 50 years who accounted for 28 % (14 patients) followed by 30 – 40 years age group, 13 patients (26%). The least number of patients in the 60 – 70 and 0-20 years age group-2 patients each (4%).
4. The Mean age is 40.6 yrs with SD 12.1 yrs. Median Age was 39.5 yrs.
5. After the 50 patients were divided based on Serum Iron Levels 27 (54%) of them were in the Normal Iron Deficient Group (A) and 23 (46%) were in the Iron Deficient Group (B).
6. Female patients far exceeded males in the iron deficient group. In both the groups A and B the number of multiparous females who suffered from cholelithiasis was more than primipara and nullipara.
7. In The Non Iron-Deficient patients (n=27)-Group A:
   * The average serum iron was 93.2± 35 micro gm/dl.
   * The Mean Biliary Cholesterol Level was

     MEAN=754.5 mg/dl SD=398.3 mg/dl
   * The Mean Serum Cholesterol Levels were 184.8 ±35 mg%

8. In the Iron-Deficient patients (n=23)-Group B:

   * The average serum iron was 26.8± 8.6 micro gm/dl.

   * The Mean Biliary Cholesterol Level was

     MEAN=1184.7 mg/dl SD=405.2 mg/dl

   * The Mean Serum Cholesterol Levels were 172±49 mg%

9. The Biliary Cholesterol values for Group A and B respectively were 754.5 ± 398.3 and 1184.7 ± 405.2 mg/dl.

10. The Biliary Cholesterol levels were significantly higher in the Iron Deficient group (Group B) than compared to the Non-Iron Deficient Group. This result was extremely statistically significant with a p value of <0.0004.

Patients with gall stone disease and low Serum Iron have higher values of biliary cholesterol, possibly implicating the role of Serum Iron in the pathogenesis of cholesterol gall stones.

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# ANNEXURE

PROFORMA

NAME: DATE OF ADMISSION:

AGE : DATE OF DISCHARGE:

SEX : ADDRESS:

IP NO.

OCCUPATION:

CHIEF COMPLAINTS:

PAST HISTORY :

PERSONAL HISTORY:

FAMILY HISTORY:

GENERAL EXAMINATION:

PULSE:BP:TEMPERATURE:

EXAMINATION OF ABDOMEN:

*INVESTIGATIONS:*

HB :TC:DC:

BLOOD UREA:SERUM CREATININE:

LFT:

SERUM IRON LEVELS:

SERUM LIPID PROFILE (Total Cholesterol):

BILIARY LIPID PROFILE (Total Cholesterol):

USG ABDOMEN:

OTHER INVESTIGATIONS:

# CONSENT FORM

## சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு :

**COMPARITIVE ANALYSIS OF BILIARY CHOLESTEROL LEVELS IN IRON DEFICIENT AND NON-IRON DEFICIENT PATIENTS OPERATED FOR GALL STONE DISEASE**

ஆராய்ச்சி நிலையம் : பொது அறுவை சிகிச்சைத் துறை
கீழ்பாக்கம் மருத்துவக் கல்லூரி
சென்னை – 600 010.

பங்கு பெறுபவரின் பெயர் : வயது :

பங்கு பெறுபவரின் எண். :

பங்கு பெறுபவரது இதனை (✓) குறிக்கவும்

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களைப் பெறவும் வாய்ப்புளிக்கப்பட்டது. ☐

நான் இவ்வாய்வின் தன்னிச்சையாகத்தான் பங்கேற்கிறேன். எந்தக் காரணத்தினாலோ எந்தக் கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்று அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்மந்தமாகவோ, இதைச் சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும்போது இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளைப் பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான முடிவுகளையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக் கொள்ளவும் அதைப் பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கிறேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். எனக்குக் கூறப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன், இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன். என் உடல் நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத நோய்க்குறி தென்பட்டாலோ உடனே அதை மருத்துவ அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம் .....இடம் ..... தேதி
கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம் ..... தேதி

ஆய்வாளரின் பெயர் .....

# MASTER CHART

| Sr. No | IP No. | NAME | AGE | SEX | Parity | Serum Iron In ug/dl | Study Group A vs B | Biliary Cholesterol Levels mg/dl | Serum Cholesterol Levels mg/dl |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 15968 | Lakshmi | 48 | F | Multi | 7.5 | B | 162.7 | 79 |
| 2 | 16058 | Ravi | 58 | M | N/A | 116.7 | A | 65.4 | 187.1 |
| 3 | 16373 | Balasaraswathy | 53 | F | Multi | 95.1 | A | 8.6 | 253.9 |
| 4 | 16406 | Machagandhi | 38 | F | Multi | 33.4 | B | 98.9 | 224.3 |
| 5 | 17004 | Girija | 21 | F | Null | 34 | B | 26.5 | 135.6 |
| 6 | 17671 | Velu | 26 | M | N/A | 95.5 | A | 121 | 142.9 |
| 7 | 17777 | Kesavan | 52 | M | N/A | 125 | A | 121.1 | 149.6 |
| 8 | 17804 | Girija | 46 | F | Multi | 27.3 | B | 133.6 | 197.2 |
| 9 | 17839 | Rejina | 39 | F | Multi | 128.3 | A | 115.9 | 164.2 |
| 10 | 18221 | Malar | 28 | F | Primi | 123.5 | A | 17.3 | 187.1 |
| 11 | 18427 | Geetha | 40 | F | Multi | 30.7 | B | 103.5 | 154.2 |
| 12 | 18561 | Omprakash | 32 | M | N/A | 103 | A | 76.8 | 244.1 |
| 13 | 18570 | Kamarunisha | 48 | F | Multi | 14.1 | B | 162.3 | 199 |
| 14 | 19049 | Ashwani | 29 | F | Multi | 75 | A | 94.2 | 167.8 |
| 15 | 19083 | Jayaraman | 63 | M | N/A | 152.1 | A | 39 | 170.5 |
| 16 | 19494 | Patchaiyammal | 32 | F | Multi | 40.3 | A | 52.9 | 200.8 |
| 17 | 19536 | Zareena | 19 | F | Null | 101.2 | A | 3.5 | 172.5 |
| 18 | 19620 | Thajunisha begum | 25 | F | Primi | 33.8 | B | 60.4 | 165.2 |
| 19 | 19668 | Geetha | 32 | F | Multi | 34.6 | B | 65.3 | 231.9 |
| 20 | 19799 | Vijayalakshmi | 57 | F | Multi | 42.3 | A | 16 | 167.1 |
| 21 | 20057 | Subramani | 39 | M | N/A | 163.7 | A | 146.7 | 208.8 |
| 22 | 20260 | Minnala | 60 | F | Multi | 27 | B | 147 | 137.1 |
| 23 | 20269 | Nallathambi | 45 | M | N/A | 96 | A | 51.4 | 230.9 |
| 24 | 20360 | Gowramma | 42 | F | Multi | 28.8 | B | 108.7 | 218.6 |
| 25 | 20589 | Alima | 30 | F | Multi | 36.3 | A | 61.4 | 222.7 |
| 26 | 20830 | Kala | 42 | F | Multi | 70.8 | A | 117.5 | 157.3 |
| 27 | 20862 | Manoranjitham | 58 | F | Multi | 73.3 | A | 65 | 156.2 |
| 28 | 21236 | Meena | 47 | F | Multi | 7.4 | B | 161.3 | 159.8 |
| 29 | 21585 | Sasikala | 30 | F | Multi | 53.5 | A | 44.9 | 198.9 |
| 30 | 21864 | Gambeeram | 60 | M | N/A | 96.2 | A | 92.5 | 206.5 |
| 31 | 22112 | Susana | 41 | F | Multi | 29 | B | 106.5 | 152.7 |
| 32 | 22113 | Moorthy | 37 | M | N/A | 40 | B | 180.6 | 203.7 |
| 33 | 23653 | Gowthami | 39 | F | Multi | 103.1 | A | 92.3 | 218.5 |
| 34 | 23801 | Rani | 50 | F | Multi | 20.5 | B | 159.4 | 155.8 |

| Sr. No | IP No. | NAME | AGE | SEX | Parity | Serum Iron In ug/dl | Study Group A vs B | Biliary Cholesterol Levels mg/dl | Serum Cholesterol Levels mg/dl |
|---|---|---|---|---|---|---|---|---|---|
| 35 | 23802 | Udaya | 28 | F | Primi | 91.4 | A | 99.2 | 147.7 |
| 36 | 24049 | Murugeshwari | 52 | F | Multi | 22.8 | B | 150.8 | 139.3 |
| 37 | 24244 | Krishnan | 48 | M | N/A | 130.6 | A | 106.1 | 184.3 |
| 38 | 24253 | Chandramathi | 68 | F | Multi | 27.2 | B | 135.7 | 269.4 |
| 39 | 24360 | Jasmine | 38 | F | Multi | 77.2 | A | 72.6 | 183.1 |
| 40 | 24766 | Sundaramma | 45 | F | Multi | 28.1 | B | 131.9 | 227.1 |
| 41 | 24885 | Mahalakshmi | 42 | F | Multi | 29.1 | B | 105.4 | 106.5 |
| 42 | 24951 | kalaiyarasi | 38 | F | Multi | 33.7 | B | 91.9 | 154.1 |
| 43 | 25508 | Sumathi | 29 | F | Primi | 64.6 | A | 43.1 | 178.3 |
| 44 | 25731 | Lavanya | 17 | F | Null | 140.1 | A | 65.3 | 172.3 |
| 45 | 26154 | Govindammal | 42 | F | Multi | 28.5 | B | 112 | 137.2 |
| 46 | 26273 | Angel | 24 | F | Primi | 32.3 | B | 57.1 | 224 |
| 47 | 26406 | Kumudha | 39 | F | Multi | 44.1 | A | 127.2 | 224.8 |
| 48 | 26577 | Sneha | 35 | F | Multi | 32.8 | B | 102 | 180.6 |
| 49 | 26613 | Sulochana | 50 | F | Multi | 14.8 | B | 161.5 | 82 |
| 50 | 26744 | Shoba | 28 | F | Primi | 77.7 | A | 120.5 | 93.3 |

## KEY TO MASTER CHART

***ABBREVIATIONS USED:***

| **Column Data** | **Interpretation** |
|---|---|
| Sex<br>M<br>F | <br>Male<br>Female |
| Parity<br>Null<br>Primi<br>Multi<br>N/A | <br>Nulliparous<br>Primiparous<br>Multiparous<br>Not-Applicable |
| Serum Iron Levels | Measured in Micro gm/dl |
| Biliary Cholesterol Levels | Measured in Milli gm/dl |
| Serum Cholesterol Levels | Measured in Milli gm/dl |
| Study Group<br>A<br>B | <br>Non-Iron Deficient Group<br>Iron Deficient Group |
| Normal Iron Range criteria used in Study | Males (60-160 μg/dl)<br>Females(35-145 g/dl) |